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L6	l1 and l5	26	L6
L5	protein hydrolysate	3581	L5
L4	l3 same protease	7	L4
L3	(soy near5 flour) same defat$6	341	L3
L2	l1 and protease$4	60	L2
L1	(soy near5 flour) and defat$6	520	L1

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L6: Entry 2 of 26 File: USPT Apr 16, 2002

DOCUMENT-IDENTIFIER: US 6372282 B1
TITLE: Method for producing a protein hydrolysate

Brief Summary Paragraph Right (1):
The present invention relates to a method for preparing a protein hydrolysate, more specifically to an enzymatically prepared protein hydrolysate.

Brief Summary Paragraph Right (2):
In the food industry protein hydrolysates may be used as food products or as additives to food products.

Brief Summary Paragraph Right (3):
Conventionally, protein hydrolysates are produced chemically by hydrolysing protein or proteinaceous material, such as for example defatted soy flour or wheat gluten, with hydrochloric acid under refluxing conditions. The resulting hydrolysates are cheap and tasty. However, chemical hydrolysis also results in the formation of chlorohydrins, such as monochlorodihydroxypropanols (MCDPs) and dichloropropanols (DCPs), the presence of which is undesirable in food products.

Brief Summary Paragraph Right (6):
To achieve maximum amino acid generation, commercial exopeptidase-rich preparations such as Flavourzyme.RTM. (NOVO Nordisk, Denmark) and various Sumizyme.RTM. preparations (Shin Nihon, Japan), contain a mixture of various endoproteases to create as many starting-points as possible for the different exopeptidases. Furthermore, these preparations contain different types of amino-terminal and carboxy-terminal exopeptidases to overcome the relatively high specificity towards particular amino acids sequences that most exopeptidases possess. As a result of their complex nature, these currently available endoprotease/exopeptidase mixtures are expensive and determine to a large extent the cost of the resulting hydrolysate. As various endoproteases are readily available as relatively pure and cost effective products, it is the complex mixture of exoproteases, which is considered essential, that is the cost determining factor. For example, WO94/25580 describes the use of at least five proteolytic components to provide a protein hydrolysate which is useful as a flavouring agent.

Brief Summary Paragraph Right (7):
The present invention relates to a method for preparing a protein hydrolysate. The method comprises contacting proteinaceous starting material, under aqueous conditions, with a proteolytic enzyme mixture which comprises only one exopeptidase. Cost-effective production of such single exopeptidase preparations may be feasible using well-documented cloning techniques.

Brief Summary Paragraph Right (10):
The present invention provides a method for preparing a protein hydrolysate which comprises contacting proteinaceous starting material, under aqueous conditions, with a proteolytic enzyme mixture which comprises only one exopeptidase. A proteolytic enzyme mixture according to the invention, i.e. comprising a single selected exopeptidase in combination with one or more endoproteases, may hydrolyse vegetable or animal protein at least as effectively as an enzyme mixture which contains several exoproteases. Since cost-effective production of such single exopeptidase preparations is feasible using well-documented cloning techniques, the method according to the invention enables the economic production of protein hydrolysates.

Brief Summary Paragraph Right (14):
Examples of protein or proteinaceous material which may be hydrolysed by the method of the invention are known to the person skilled in the art and include vegetable proteins such as soy protein, wheat gluten, rape seed protein, pea protein, alfalfa protein, sunflower protein, zein, and animal derived protein such as casein, egg white, whey protein and meat protein. As some vegetable proteins such as wheat gluten have low solubilities under the pH conditions used, chemically treated versions of such protein sources provide another interesting group of substrates. In a preferred embodiment of the invention, the proteinaceous material used is defatted soy flour or wheat gluten.

Detailed Description Paragraph Right (2):
Finely milled and non-heat treated defatted soy flour (type Cargill 200/80) was obtained from Cargill (Amsterdam, Netherlands).

Detailed Description Paragraph Right (16):
Under pH and temperature conditions feasible for large scale production, a suspension of soy flour in water was incubated with different mixtures of endoprotease and exopeptidases. To allow testing of small quantities of highly purified enzymes under constant pH conditions and complete mixing, the dry matter content of the suspension was limited to 10% (w/w) soy flour in buffer. Enzymes added were standardised on enzyme protein per quantity of soy flour. After incubation, the degree of hydrolysis was determined by comparing the total quantity of solubilized amino acids with the total quantity of amino acids present.

Detailed Description Paragraph Right (22):
Vegetable protein sources, and soy flour in particular, are cheap starting materials for protein hydrolysates. However, soy flour contains a number of components which could have a negative impact on the taste, colour and nutritional aspects of the final hydrolysate. For example, defatted soy flour contains, in addition to 50% of protein, approximately 13% of polymeric sugars, 13% of oligomeric sugars and 5% of starch. Enzymatic hydrolysis of each of these compounds can lead to appreciable concentrations of highly reactive aldose sugars like glucose, galactose and xylose. In the presence of high concentrations of free amino acids, and catalysed by various processing steps, such sugars may lead to undesirable Maillard products.

Detailed Description Paragraph Right (29):
In Examples 1-2 it has been demonstrated that in hydrolysing soy flour the type of endoprotease selected plays a role in hydrolysis. Quite surprising was that under the selected industrial incubation conditions, i.e. pH 5-5.5, 55.degree. C., mixtures containing only a single exoprotease could be shown to be superior to multi-enzyme preparations like Flavourzyme.RTM. or Sumizyme.RTM. FP in terms of amino acid release.

Detailed Description Paragraph Right (30):
In this Example we will demonstrate that this superiority of single exoprotease incubations is not limited to combinations of carboxypeptidase Y with soy flour. The data provided in Table 5 show that also combinations of vital wheat gluten with carboxypeptidase Y, whey protein with CPD-III and casein with CPDS.sub.1 yield superior hydrolysis kinetics. In all of these examples the single exoprotease was combined with Maxatase.RTM. to generate the peptides which serve as a substrate for the pure exoproteases.

Detailed Description Paragraph Right (31):
Apart from soy flour, wheat gluten represents an economically attractive source of amino acids. Wheat gluten is known to be exceptionally rich in glutamine containing stretches so that enzymatic hydrolysis of gluten can be expected to yield relatively large quantities of free glutamine, which is a desirable precursor of glutamic acid. Both the enzymatic and chemical conversion of glutamines released by enzymatic hydrolysis into glutamic acid and hence MSG, has been adequately described in the literature.

Detailed Description Paragraph Right (36):
To test the performance of a heat stable aminopeptidase in terms of releasing amino-acids, chromatographically pure AP II enzyme was incubated with purified

Maxatase.RTM. and defatted soy flour at 70.degree. C. and pH 7.4. The results of the experiments obtained with AP II shown in Table 7 clearly demonstrate that under the high temperature conditions applied the performance of the single exoprotease is superior to the complex mixture of exoproteases present in a commercial preparation.

Detailed Description Paragraph Left (2):
Hydrolysis of Soy Flour using Various Enzyme Mixes

Detailed Description Paragraph Left (3):
Conditions: 10% soy flour in 0.25 M sodiumcitrate, pH 5.2 at 55.degree. C.; per mixture 2% enzyme protein on soy flour was added.

Detailed Description Paragraph Left (12):
Hydrolysis of Soy Flour using Heat-stable Enzyme

Detailed Description Paragraph Left (13):
Conditions: 10% w/w soy flour in 0.25 M sodium phosphate, pH 7.4 at 70.degree. C.; per mixture incorporating exoproteolytic activity 2% enzyme protein (i.e. 4 mg) on soy flour was added.

CLAIMS:

1. A method for preparing a protein hydrolysate from a proteinaceous material which method comprises contacting said material under aqueous conditions with a proteolytic enzyme mixture which comprises at least one endopeptidase and to which has been added a single pure exopeptidase.

18. A method according to claim 17 wherein the vegetable protein is defatted soy flour or optionally deamidated wheat gluten.

22. A protein hydrolysate obtainable by the methods of 21.

23. A food product containing a protein hydrolysate according to claim 22.

24. A process for preparing a food product comprising (i) preparing a protein hydrolysate according to claim 22 and (ii) formulating a food product using the hydrolysate.

L6: Entry 4 of 26 File: USPT Nov 6, 2001

DOCUMENT-IDENTIFIER: US 6313273 B1
TITLE: Soy proteins and methods for their production

Abstract Paragraph Left (1):
A high quality soy protein concentrate (SPC) was produced by a process of enzyme treatment combined with ultrafiltration. Soy flour, the starting material, was enzymatically treated with commercial pectinases and diafiltered with a porous stainless steel ultrafiltration system. The resulting product had reduced levels of physic acid and nucleic acids due to contaminant phytase and nuclease activity in the pectinase enzymes. The functionality of the SPC was improved due to increased solubility compared to conventional soy isolates produced by acid precipitation. High performance liquid chromatography gel filtration profiles indicated that the proteins in the SPC remained intact. The SPC also had reduced flavor when compared to the original soy flour according to gas chromatography flavor profiles and sensory evaluation.

Brief Summary Paragraph Right (3):
Raw soybeans and soy flour are characterized by odors described as green, grassy, bitter and beany and are therefore undesirable to many consumers. Volatile compounds contributing to soy flavor have been identified in numerous publications over the past 4 decades. A review of soy flavor (MacLeod, G. & Ames, J., (1988) Soy flavor and its improvement, CRC Critical Reviews in Food Science and Nutrition. 27 (4): 219-400) stated that 334 separate volatile compounds had been identified from soybeans, flours, concentrates, isolates, and textured soy proteins. The compounds identified were from the chemical classes of aliphatic hydrocarbons, alicyclic hydrocarbons, terpenoids, aliphatic alcohols, aliphatic aldehydes, aliphatic ketones, alicyclic ester, aliphatic ethers, aliphatic amines, aliphatic nitrile, chlorine containing compounds, benzenoids, sulfur compounds, benzenoids, sulfur compounds, furanoids, thiophenoids, pyrroles, pyridine, pyrazines, and thiazoles.

Brief Summary Paragraph Right (9):
Phytic acid is inositol hexaphosphoric acid, and is part of a large class of compounds that influence the functional and nutritional properties of foods. The phytic acid content of soybeans is reported to be between 1.0 and 1.47% of the dry weight. This is about 60% of the total phosphorus in the soybean. The amount of phytic acid in soy flour has been reported to be as high as 2.24% (w/w). Phytate forms complexes with proteins and with mono- and divalent cations. Therefore, phytate in food components may cause the proteins and minerals to have limited bioavailability. Since phytate is associated with the proteins, protein products also have high levels of phytate.

Brief Summary Paragraph Right (13):
Nucleic acids are another substance that would be desirable to remove from soy protein. Infant formula incorporating soy protein produced via current commercial processes has significantly higher levels of nucleic acids than human breast milk. Defatted soybeans reportedly contain 1.66% ribonucleic acid. Nucleotides contain a nitrogenous base (pyrimidine or purine), a pentose and a phosphate. A nucleoside is a nitrogenous base and a pentose without a phosphate (Lehnigher, A. L., Nelson, D. L., Cox, M. M. (1993) Principles of Biochemistry. New York: Worth Publishers)

Brief Summary Paragraph Right (18):
Soy proteins are typically in one of three forms when consumed by humans. These include flour (grits), concentrates, and isolates. All three types are made from defatted soybean flakes. Flours and grits contain at least 50% protein and are

prepared by milling the flakes. Soy protein concentrates contain at least 70% protein on a dry weight basis. Concentrates are made by repeatedly washing the soy flakes with water, which may optionally contain low levels of food grade alcohols or buffers. The effluent from the repeated washings is discarded and the solid residue is dried, thereby producing the desired concentrate. The yield of concentrates from the starting material is approximately 60-70%.

Brief Summary Paragraph Right (19):
Soy protein isolates contain a minimum of 90% protein on a dry weight basis. Isolates are made by extracting the soy flour with a dilute alkali (pH <9) and centrifuging. The extract is adjusted to pH 4.5 with a food grade acid such as sulfuric, hydrochloric, phosphoric or acetic acid. At a pH of 4.5, the solubility of the proteins are at a minimum so they will precipitate out. The acid precipitated protein curd is centrifuged, washed, neutralized and spray dried to produce the soy protein isolate. The yield of the isolate is 30% of the original soy flour and 60% of the protein in the flour.

Brief Summary Paragraph Right (20):
Due to the potential for improving the properties of soy protein, research has been carried out on alternative ways of preparing soy flours, concentrates and isolates. Some of this research has focused upon ultrafiltration. Ultrafiltration is a method used to separate molecules based on molecular size or shape. The membrane acts as a selective barrier. A solution is pumped through a semi-permeable membrane. The membrane retains compounds higher in molecular weight while smaller molecules and water pass through the membrane.

Brief Summary Paragraph Right (25):
Omasaiye, O., Cheryan, M., Matthew, E., (1978) Removal of oligosaccharides from soybean water extracts by ultrafiltration, Journal of Food Science, 43: 354-360, made a full-fat soy protein concentrate by ultrafiltration. Defatted soy flour was the typical starting material for soy protein concentration processing. In this study, soybeans were the starting material. Soybean water extracts were fed into the ultrafiltration system for continuous diafiltration. The composition of the diafiltered product was 58.26% protein, 33.56% fat, 0.77% oligosaccharides, 3.43% ash and 3.98% other compounds.

Brief Summary Paragraph Right (28):
Nicholas, D. J., Cheryan, M. (1981) Production of soy isolates by ultrafiltration: Factors affecting yield and composition, Journal of Food Science, 46: 367-372, studied the factors affecting the yield and composition of soy protein isolates during an ultrafiltration process. The starting material was an extract of defatted soy flour. The molecular weight cut-off of the membrance was 50,000. In order for the ultrafiltration step to produce a product with a protein content of 90%, over 80% of the non-protein solutes needed to be removed. The starting material had a protein content of 65%. The highest protein content obtained was 84% on a dry weight basis. Therefore, the ultrafiltration step did not fractionate the compounds to the degree necessary to produce a soy protein isolate. Pumping problems and severe membrance fouling were sited as problems. As observed in other studies (Omosaiye and Cheryan, (1979b), supra) the mineral content did not decrease according to predicted permeability of the membrane, perhaps due to mineral-protein binding. The highest protein yield obtained was 86%.

Brief Summary Paragraph Right (32):
In its more preferred embodiments, the process is directed to the production of soy protein concentrates and isolates. Typically a soy flour will be contacted with commercial grade enzymes (pectinases) under conditions suitable for an enzymatic reaction. The product of the enzymatic reaction will be pumped directly under pressure into a tubular housing unit which contains one or more metalic oxide ultrafiltration membranes. Typically these ultrafiltration membranes are secured along the inside surfaces of the housing unit. After the ultrafiltration is completed, the resulting retentate is diluted with an aqueous solution and subjected to a diafiltration in the same ultrafiltration unit. The aqueous solution may be added continually or in a batchwise manner.

Brief Summary Paragraph Right (36):

In addition to having reduced levels of phytate, isoflavone, and nucleic acids; the soy protein has superior emulsifying capacities. Soy protein produced via prior art methods are exposed to acidic washes. The acidic treatment has a tendency to denature the protein and reduce its capability to serve as an emulsifier in infant formula. The soy protein of this invention has a water hydration capacity of about 2 to about 5 and more preferably about 2.6% which is not different from soy flour (see Quinn, J. R. and Paton, D. 1979, A practical measurement of water hydration capacity of protein materials. Cereal Chem. 56: 38-40 for methodology). Surface hydrophobicity of soy protein produced via the invention is typically no greater than about 30, is more typically in the range of about 15-25 and more preferably about 20 (see Hayakawa, S. and Nagai, S. 1985, Relationships of hydrophobicity and net charge to the solubility of milk and soy proteins. Journal of Food Science 50: 486-491 for methdology). Nitrogen solubility of the soy protein produced via this invention, when measured at a pH of 7.0 is typically no less than about 40 w/w%, more typically ranges from about 50-70 w/w% and more preferably is no less than about 57 w/w% (see Bera, M. B. and Mukherjee, R. K. 1989, Solubility, emulsifying and foam properties for rice bran protein concentrates. Journal of Food Science 50: 142-145 for methodology). Soy protein produced via the methodology of this invention will have an emulsifying capacity of no greater than about 7 meters square per gram (m.sup.2 /g), more typically about 4-7 m.sup.2 /g and most preferably about 6.0 m.sup.2 /g (see Pearce, K. N. and Kinsella, J. E. 1978. Emulsifying properties of proteins; Evaluation of a turbidimetric technique Journal of Agricultural Food Chem. 26: 716-723 for a description of the methodology) which is not significantly different from that of soy flour and commercial isolate. The stability of the emulsion formed is also important in determining the emulsifying properties. Soy protein produced via this invention has an emulsion stability index of greater than 30 m.sup.2 /g, more typically from 30-50 m.sup.2 /g and more preferably about 40 m.sup.2 /g.

Drawing Description Paragraph Right (2):
FIG. 2 is a High Performance Liquid Chromatogram--Gel Filtration profile of Soy Flour, Diafiltered Retentate and Permeate.

Drawing Description Paragraph Right (3):
FIG. 3 is a Gas Chromatogram (GC) of Soy Flour and Permeate.

Drawing Description Paragraph Right (4):
FIG. 4 is a GC of Soy Flour and Diafiltered Retentate.

Drawing Description Paragraph Right (5):
FIG. 5 is a GC of Soy Flour, Filtered Retentate and Diafiltered Retentate.

Drawing Description Paragraph Right (6):
FIG. 6 is a GC of Soy Flour, Diafiltered Retentate and Permeate.

Drawing Description Paragraph Right (7):
FIG. 7 is a spider plot describing the aroma of Soy Flour, Membrane Soy Concentrate and Commercial Soy Isolate.

Drawing Description Paragraph Right (8):
FIG. 8 is a spider plot describing the flavor of Soy Flour, Membrane Soy Concentrate (produced via the invention) and Commercial Soy Isolate.

Drawing Description Paragraph Right (10):
FIG. 10 is a graph showing the comparison of nitrogen solubility among Soy Flour, Membrane Soy Concentrate and Commercial Soy Isolate in the pH range of 3 to 10.

Drawing Description Paragraph Right (11):
FIG. 11 is a bar graph comparing the emulsifying properties of Bovine Serum Albumin, Soy Flour, Membrane Soy Concentrate and Commercial Soy Isolate.

Detailed Description Paragraph Right (2):
As noted above, the present invention is directed to a multistep process for isolating and purifying soy proteins. The soy protein is isolated from the soybean. The soybean is an excellent source of high quality protein, where about 38% to 40% of the soybean is protein. Briefly (as shown in Scheme I), the processing of soybeans involves the

extraction of the oil from the dehulled, and cracked soybeans leaving the defatted soybean flakes. ##STR2##

Detailed Description Paragraph Right (3):
The defatted soybean flakes are typically milled into flours. As described above, they may be further processed into protein concentrates or isolates. One aspect of this invention is directed to methods for the production of these concentrates and isolates.

Detailed Description Paragraph Right (4):
Typically defatted soy bean flakes or soy flours will be the source of soy protein in the inventive process. However soy concentrate may be utilized as well.

Detailed Description Paragraph Right (10):
The soy flour (or other source of soy protein) will be diluted with an aqueous solution in the reaction vessel. Typically only water will be used, but dilute alcohol may also be used. The quantity of soy flour can vary widely. Typically the soy source will be present in the reaction vessel in an amount ranging from about 5% to about 12.5% and more preferably about 10%, based upon weight. The quantity of enzyme can vary, but will typically be present in an amount of at least 0.3% v/v and more preferably about 0.3% to about 0.9% v/v based upon the phytase activity of the enzyme and the quantity of the soy material. The enzyme reaction will be carried out at a temperature of about 84.50.degree. F. to about 1220.degree. F. and more preferably about 98.60.degree. F. to about 107.60.degree. F. The enzymatic reaction will be allowed to continue for a period of time of at least about three hours and optionally longer. At the conclusion of the enzymatic reaction, the solution is pumped directly into the ultrafiltration apparatus.

Detailed Description Paragraph Right (11):
The next step in the process is the ultrafiltration of the enzyme treated soy flour solution. The ultrafiltration will be carried out using techniques generally known in the art. A detailed discussion of ultrafiltration techniques and apparatuses can be found in the "Ultrafiltration Handbook" by Munir Cheryan, Technomic Publishing Co. Lancaster, Pa. (which is hereby incorporated by reference).

Detailed Description Paragraph Right (25):
The soy protein produced by the process above differs from that produced in the prior art. It has reduced levels of phytate. For example, soy flour (the typical starting material in the inventive process), contains 21-22 mg of phytate per gram of soy protein. Soy protein produced according to the invention will contain no more than about 5 mg of phytate and more preferably no more than about 1.6 to 1.7 mg of phytate. Soy flour typically contains about 7 to 8 gram of ribonucleic acids per kilogram of soy flour. Soy protein produced via the instant invention will contain no more than about 0.3 to about 0.4 grams of ribonucleic acids per kilogram of soy protein.

Detailed Description Paragraph Right (26):
The soy proteins of this invention also have enhanced solubility compared with those of the prior art. Soy protein that is precipitated from soy flour via acidic conditions (ie. a pH of less than 4.5) has a nitrogen solubility of 17 w/w % in water at room temperature. The soy proteins of this invention, typically have a solubility of at least 40 w/w % in water, at room temperature, at a pH of 7.0 and more typically about 55 w/w % under comparable conditions.

Detailed Description Paragraph Right (27):
Soy protein that is precitated from soy flour via acidic conditions (i.e., a pH of less that 4.5) has a surface hydrophobicity of 36.77 as determined by Hayakawa et al., supra. The soy proteins of this invention will have a hydrophobicity of no more than about 30 and more typically about 20.

Detailed Description Paragraph Right (28):
The soy protein produced via the instant invention has superior emulsifying capacities. Soy protein that is precipitated from soy flour via acidic conditions (ie. a pH of less than 4.5) has an emulsifying activity index of about 8.2 m.sup.2 /gram and a stability index of about 27m.sup.2 /gram. The soy protein produced according to this invention will have an emulsifying activity index of no greater than about

6m.sup.2 /gram and a stability index of about 40 m.sup.2 /gram.

Detailed Description Paragraph Right (37):
1000 grams of defatted soy flour was diluted in 20 liters of distilled water to give a 5% w/v solution to which 60 milliliters of the enzyme pectinase was added at a ratio of 0.3% v/v. The pectinase was obtained from Sigma Chemical Company of St. Louis, Mo. having a declared activity of 11.8 units/ milligram protein. (One unit will liberate 1.01 .mu.mole of galacturonic acid per min at pH 4.0 at 25.degree. C.). The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-42.degree. C. for three hours. The solution was then pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 cm.times.1.57 cm i.d. per membrane). The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration was 2.88 gallons/sq. ft./day at 950.degree. F. The inlet pressure was 29.5 psi. Ten liters of permeate collected was labeled as the microfiltered permeate at which time the concentration of solids was 2x. The flux at the end of microfiltration was 0.81 gallons/sq.ft./ day at 102.2.degree. F. and the inlet pressure was 40 psi. This was designated as the end of microfiltration. Diafiltration that follows microfiltration does not result in any further concentration of solids. Ten liters of water equal to the volume of microfiltered permeate collected as added back to the kettle and filtration was allowed to continue through the membranes until ten liters of diafiltered permeate was collected. The permeate flux immediately after the addition of ten liters of water (i.e., at the start of diafiltration) was 2.00 gallons/sq.ft./day at 86.degree. F. and the inlet pressure was 34 psi. At the end of diafiltration the permeate flux was 0.96 gallons/sq. ft./day at 105.80.degree. F. and the inlet pressure was 44 psi. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the permeate (6.23) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was then centrifuged at 2000.times.g for 20 minutes to remove the insoluble solids. The supernatant was the freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (38):
1000 grams of defatted soy flour was diluted in 20 liters of distilled water to give a 5% w/v solution to which 180 milliliter of the enzyme Crystalzyme 100XL was added at a ratio of 0.9% v/v. The crystalzyme was obtained from Valley Research, Inc., South Bend, IN having a declared activity of 110,000 Apple Juice Depectinising Units (AJDU) units/gram protein. The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-42.degree. C. for three hours. The solution was then pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 cm.times.1.57 cm i.d. per membrane). The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration was 4.32 gallons/sq.ft./day at 98.6.degree. F. The inlet pressure was 25 psi. Ten liters of permeate collected was labeled as the microfiltered permeate at which time the concentration of solids was 2x. The flux at the end of micro filtration was 0.72 gallons/sq.ft./day at 118.40.degree. C. and the inlet pressure was 30 psi. This was designated as the end of microfiltration. Diafiltration that follows microfiltration does not result in any further concentration of solids. Ten liters of water equal to the volume of microfiltered permeate collected was added back to the kettle and filtration was allowed to continue through the membranes until ten liters of diafiltered permeate was collected. The permeate flux immediately after the addition of 10 liters of water (i.e., at the start of diafiltration) was 2.16 gallons/sq.ft./day at 122.degree. F. and the inlet pressure was 40 psi. At the end of diafiltration the permeate flux was 1.20 gallons/sq.ft./day at 122.degree. F. and the inlet pressure was 30 psi. The pump was shut off after diafiltration and the retentate was collected from the kettle for further processing. The pH of the permeate (6.20) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was then centrifuged at 2000.times.g for 20 minutes to remove the insoluble solids. The supernatant was then freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (39):
500 grams of defatted soy flour was diluted in 10 liters of distilled water to give a 5% w/v solution to which no enzyme was added and this was treated as control. The enzyme treatment was stirred in a steam jacketed kettle whose temperature was maintained between 37-420.degree. C. for three hours. The solution was then pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 cm.times.1.57 cm i.d. per membrance). The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration was 3.16 gallons/sq.ft./day at 950.degree. F. The inlet pressure was 14 psi. Five liters of permeate collected was labeled as the microfiltered permeate at which time the concentration of solids was 2.times.. The flux at the end of microfiltration was 0.93 gallons sq. ft./day at 102.2.degree. F. and the inlet pressure was 20 psi. This was designated as the end of microfiltration. Diafiltration that follows microfiltration does not result in any further concentration of solids. Five liters of water equal to the volume of microfiltered permeate collected was added back to the kettle and filtration was allowed to continue through the membranes until five liters of diafiltered permeate was collected. The permeate flux immediately after the addition of five liters of water (i.e., at the start of diafiltration) was 2.16 gallons/sq.ft./day at 86.degree. F. and the inlet pressure was 35 psi. At the end of diafiltration the permeate flux was 0.77 gallons/sq.ft./day at 105.8.degree. F. and the inlet pressure was 13 psi. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the permeate (6.20) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was then centrifuged at 2000|.times.g for 20 minutes to remove the insoluble solids. The supernatant was then freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (40):
The freeze dried flaky powder obtained after the ultrafiltration used to concentrate the soy proteins from defatted soy flour was pulverized to a fine powder using a mortar and pestle. The freeze dried retentate from the enzyme treatments and membrane processing, soy flour and permeate collected were analyzed for protein. The protein content was calculated using a conversion factor of 6.25 to convert the nitrogen content estimated by the Microkjeldahl analysis as described in AOAC Section 47.021, 1975 and are presented in Table 1.

Detailed Description Paragraph Right (41):
As seen in Table 1 the protein content of the diafiltered retentate was only 56.2% but adjusting the pH to 9.0 resulted in a protein content of 76.7% and 78.5% respectively. This example therefore suggest that increasing the pH is necessary to enhance the solubility and recovery of soy proteins. Also, the permeate contained less that 0.5% suggesting that the nitrogen may be non protein nitrogen released from nucleic acids and that the rejection of the soy bean proteins by the membranes during microfiltration and diafiltered retentate was nearly 100%. From the results of Table 1 it is clear that the soy proteins from soy flour are concentrates based on the definition that a soy protein concentrate should contain at least 70% protein on a dry weight basis.

Detailed Description Paragraph Right (42):
HPLC Gel filtration was used to determine the molecular weight profile of soy flour, permeate and retentate samples. The standard proteins used for molecular weight comparison included apoferritin (MW 443,000), .beta. amylase (MW 200,000), bovine serum albumin (MW 66,000), ovalbumin (MW 43,000) .alpha. lactalbumin (MW 14,200) and tryptophan (MW 204). The retention times of the standards are given in Table 2.

Detailed Description Paragraph Right (43):
The HPLC profiles of proteins from soy flour, diafiltered retentate and permeate are shown in FIG. 2. The retention times for peaks in the soy flour and retentate were the same but some changes in peak areas were observed. This indicated that the major soy proteins were intact in the retentate. The first peaks in the permeate had a retention time around 40 minutes which corresponds to a molecular weight of 6500 daltons. This means that proteins with molecular weights greater than 6500 were retained by the membrane whereas proteins with molecular weights less than 6500 passed through the

membrane into the permeate.

Detailed Description Paragraph Right (44):
The compositional analysis constituting of protein, carbohydrate, ash and moisture soy flour, commercial soy protein isolate and the two enzyme treated membrane soy ncentrates (MSC) was determined and the results presented in Table 2.

Detailed Description Paragraph Right (45):
Several compounds have been identified to contribute to soy flavor and odor that ave been characterized as green, grassy, bitter and beany. The typical flavor of soy has herefore been a critical factor and has limited its extensive use in the United States and Europe. The volatile compounds from soy flour, microfiltered retentate and diafiltered retentate was extracted in 50% methanol and subjected to gas chromatographic analysis. The permeate samples did not need extraction prior to gas chromatography. FIG. 3 shows that the soy flour and permeate had similar profiles with major peaks around 6, 10, and 21 minutes. FIG. 4 shows that the diafiltered retentate had smaller peaks and reduced area under the peaks, as compared to soy flour, representing a reduction of flavor. FIG. 5 shows the comparison of the GC profiles of soy flour, filtered retentate and diafiltered retentate and it was apparent that microfiltration alone resulted in only a slight reduction in the volatile components. FIG. 6 shows the comparison of the GC profiles of soy flour, diafiltered retentate and permeate and it was apparent that that volatiles removed during microfiltration and diafiltration were indeed being lost in the permeate. Statistical analysis ($p<0.001$) on the peak areas of the GC profiles showed a significant difference between that of the microfiltered and the diafiltered retentate. The results of this example suggest that microfiltration alone was not effective in removing the flavor from soy flour and in fact diafiltration was necessary to significantly reduce the volatile components in soy flour and that the flavor reduction after diafiltration was nearly 90% based on the mean peak areas in the profiles.

Detailed Description Paragraph Right (46):
While Example 7 based GC analysis suggest the effective removal of flavor compounds after microfiltration and diafiltration, the importance of sensory evaluation cannot be stressed enough. Sensory evaluations for aroma and flavor were completed for soy flour, commercial soy isolate and concentrate made with pectinase and crystalzyme. The responses for first detected aroma included beany, corn meal, musty and toasted while the responses for the first detected flavor included beany, bitter, chalky and astringent. The results of this sensory evaluation are presented in Table 3.

Detailed Description Paragraph Right (47):
From the scores in Table 3 it is clear that the general aroma and flavor differences among soy flour, soy isolate and the two membrane concentrates were noticeable to untrained human subjects. Based on this information, a more detailed descriptive sensory evaluation of soy flour, commercial soy isolate and the membrane soy concentrate processed with crystalzyme was undertaken using trained human subjects. The panelists were chosen based on their stability to identify the four basis tastes of sweet, sour, salty and bitter. The panelists were then asked to evaluate the aroma and flavor using descriptors which had been gathered from literature and preliminary discussions with the panelists. The use of standard samples helped to achieve agreement among the panelists on the definitions and relative importance of each descriptor. The chosen aroma descriptors included wheat flour like, raw soybean like, green bean like and toasted grain like. Flavor by mouth descriptors included wheat flour like, raw soybean like, green bean like, toasted grain like, sweet and bitter. FIG. 7 shows that the membrane soy concentrate was evaluated to possess a `toasted grain` aroma and flavor while FIG. 8 shows that the membrane soy concentrate possessed the least `soy bean` taste. This example therefore suggests that the absence of soy volatiles believed to contribute to the typical soybean aroma and flavor are perceived by both trained and untrained human panelists.

Detailed Description Paragraph Right (48):
Solutions of varying solids concentration in three different batches were used in the production of membrane soy concentrate so as to be able to optimize the microfiltration and diafiltration process outlined for the concentration of soy proteins. Batch I used 9.9 pounds of defatted soy flour diluted in 198 pounds of water so as to give a concentration of 5% w/w in a steam jacketed kettle to which 810

milliliters of the enzyme Crystalzyme 100XL was added at a ratio of 0.9% v/v. The crystalzyme was obtained from Valley Research, Inc., South Bend, IN having a declared activity of 110,000 AJDU units/gram protein. The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-420.degree. C. for three hours. The solution was then pumpted through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 inches.times.0.72 inches i.d. per membrane). Two such modules were used in parallel connection. In addition two single pass tubular microfiltration membranes (60 inches.times.1.25 inches i.d. per membrane) were also used in conjunction so as to increase the surface area and capacity. The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration process was 47.93 gallons/sq.ft./day at 104.degree. F. The inlet pressure was 56 psi and the outlet pressure was 34 psi. 90 pounds of water was collected a the permeate end to mark the end of microfiltration. The permeate flux at the end of microfiltration was 36.20 gallons Isq.ft./day at 119.degree. F. The inlet pressure was 74 psi and the outlet pressure was 50 psi. Diafiltration was continued as a continuous feed and bleed process wherein 100 pounds of distilled water was added in three batches of 35 pounds, 35 pounds and 30 pounds respectively. The permeate flux at the start of diafiltration was 30.93 gallons/sq.ft./day at 112.degree. F. The inlet and outlet pressures were 74 psi and 50 psi. The collection of 100 pounds permeate marked the end of diafiltration. The permeate flux was 27.12 gallons/ sq.ft./day at 118.degree. F. The inlet and outlet pressures were 76 psi and 52 psi respectively. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the retentate (6.20) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The supernatant was then freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (49):
Batch II used 19.8 pounds of defatted soy flour diluted in 198 pounds of water so as to give a concentration of 10% w/w in a steam jacketed kettle to which 810 milliliters of the enzyme Crystalzyme 100XL was added at a ratio of 0.9% v/v. The crystalzyme was obtained from Valley Research, Inc., South Bend, Ind. having a declared activity of 110,000 AJDU units/gram protein. The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-420.degree. C. for three hours. The solution was then pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 inches.times.1.25 inches i.d. per membrane) were also used in conjunction so as to increase the surface area and capacity. The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration process was 45.77 gallons/sq.ft./day at 105.degree. F. The inlet pressure was 46 psi and the outlet pressure was 28 psi. 90 pounds of water was collected at the permeate end to mark the end of microfiltration. The permeate flux at the end of microfiltration was 24.18 gallons/sq.ft./day at 119.degree. F. The inlet pressure was 55 psi and the outlet pressure was 30 psi. Diafiltration was continued as a continuous feed and bleed process wherein 100 pounds of distilled water was added in three batches of 35 pounds, 35 pounds and 30 pounds respectively. The permeate flux at the start of diafiltration was 16.78 gallons/sq.ft./day at 110.degree. F. The inlet and outlet pressures were 55 psi and 30 psi. The collection of 100 pounds permeate marked the end of diafiltration. The permeate flux was 11.69 gallons/sq.ft./day at 119.degree. F. The inlet and outlet pressures were 70 psi and 46 psi respectively. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the retentate (6.25) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was then centrifuged at 2000.times.g for 20 minutes to remove the insoluble solids. The supernatant was then freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (50):
Batch III used 22.6 pounds of defatted soy flour diluted in 180.8 pounds of water so as to give a concentration of 12.5% w/w in a steam jacketed kettle to which 739.6 milliliters of the enzyme Crystalzyme 100XL was added at a ratio of 0.9% v/v. The crystalzyme was obtained from Valley Research, Inc., South Bend, Ind. having a declared activity of 110,000 AJDU units/gram protein. The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-420.degree.

.C. for three hours. The solution was the pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 inches.times.0.72 inches i.d. per membrane). Two such modules were used in parallel connection. In addition two single pass tubular microfiltration membranes (60 inches.times.1.25 inches i.d. per membrane) were also used in conjunction so as to increase the surface area and capacity. The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. The permeate flux at the start of the microfiltration process was 29.51 gallons/sq.ft./day at 106.degree. F. The inlet pressure was 80 psi and the outlet pressure was 43 psi. 90 pounds of water was collected at the permeate end to mark the end of microfiltration. The permeate flux at the end of microfiltration was 18.75 gallons/sq.ft./day at 116.degree. F. The inlet pressure was 81 psi and the outlet pressure was 53 psi. Diafiltration was continued as a continuous feed and bleed process wherein 90 pounds of distilled water was added in three batches of 30 pounds each. The permeate flux at the start of diafiltration was 16.44 gallons/sq.ft./day at 109.degree. F. The inlet and outlet pressures were 78 psi and 50 psi. The collection of 90 pounds permeate marked the end of diafiltration. The permeate flux was 5.86 gallons/sq.ft./day at 1180.degree. F. The inlet and outlet pressures were 80 psi and 52 psi respectively. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the retentate (6.00) was adjusted to pH 9.0 with few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was the centrifuged at 2000.times.g for 20 minutes to remove the insoluble solids. The supernatant was then freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (53):
19.8 pounds of defatted soy flour was diluted in 198 pounds of distilled water to give a 10% w/w solution to which 810 milliliters of the enzyme Crystalzyme 100XL was added at a ratio of 0.9% v/v. The crystalzyme was obtained from Valley Research, Inc., South Bend, Ind. having a declared activity of 110,000 AJDU units/gram protein. The enzyme treatment was carried out in a steam jacketed kettle whose temperature was maintained between 37-42.degree. C. for three hours. The solution was then pumped through a membrane system using three porous stainless-steel tubular microfiltration membranes (60 inches.times.0.72 inches i.d. per membrane). Two such modules were used in parallel connection. In addition two single pass tubular microfiltration membranes (60 inches.times.1.25 inches i.d. per membrane) were also used in conjunction so as to increase the surface area and capacity The membranes were supplied by Graver Separations, Inc., Seneca, S.C. The retentate was returned to the steam jacketed kettle and the permeate was collected as shown in FIG. 1. 90 pounds of water was collected a the permeate end to mark the end of microfiltration. Diafiltration was continued as a continuous feed and bleed process wherein 100 pounds of distilled water was added in three batches of 35 pounds, 35 pounds and 30 pounds respectively. The pump was shut off after diafiltration and the retentate was collected for further processing. The pH of the retentate was adjusted to pH 9.0 with a few drops of 50% sodium hydroxide and stirred continuously to increase protein solubility. The solution was then centrifuged at 2000.times.g for 20 minutes to remove the insoluble solids. The supernatant was the freeze dried to obtain a flaky powder that was used for further analysis.

Detailed Description Paragraph Right (56):
Yields and recovery form an important characteristic while establishing the feasibility of a process. The theoretical yields were calculated based on the protein content of the soy flour using a mass balance ratio between the protein content and the mass of the different processing fractions which include microfiltered permeate, diafiltered permeate and the retentate. The percent distribution of protein in the different fractions of the five batches processed with 1 0% w/w solids concentration as outlined in Example 9 are calculated and presented in Table 7.

Detailed Description Paragraph Right (59):
The approximate water hydration capacity is defined as grams of water bound per gram of dry protein and describes water-protein interactions. Information on the hydration capacity is important since water is an important constituent of all food systems. The water hydration capacity of soy flour, membrane soy concentrate and commercial isolate (Supra 1610) was determined by the method outlined by Quinn and Paton (1979), supra. Surface hydrophobicity is another water-protein interaction that defines that portion

of the non-polar surface of the protein that makes contact with the surrounding bulk water. Surface hydrophobicity of the three soy protein samples were evaluated by the method outlined by Hyakawa and Nagai (1985), supra. The results of water hydration capacity an surface hydrophobicity and presented in Table 8.

Detailed Description Paragraph Right (60):
Ultrafiltration in the case of the membrane soy concentrate does not seem to have disrupted the structures so as to bring about an increase in the hydration capacity. Acid modification in the commercial soy isolate seems to have contributed to a greater water hydration capacity and seems to have unfolded the protein molecule to a large extent resulting in increased exposure of hydrophobic groups to the probe. Statistical analysis of the nitrogen solubility means indicated that the isolate had the least solubility, irrespective of the influence of pH. Soy flour showed the highest solubility with the membrane soy concentrate following a lesser but similar pattern to that of soy flour. This example suggest that membrane processing used to concentrate soy proteins seems to leave the protein molecule intact with little denaturation.

Detailed Description Paragraph Right (61):
Emulsions are dispersions of one liquid in another and are of two types viz., oil in water e.g., milk and milk products and water in oil e.g. butter and margarine. Proteins are the emulsifiers of choice for oil in water emulsions because hey are edible and surface active. Emulsifiers are evaluated both in terms of emulsifying activity and emulsifying stability because an emulsifier is important to for an emulsion and also stabilize the emulsion after it has been formed. The emulsifying properties of soy flour, membrane soy concentrate and commercial soy isolate were evaluated by the method of Pearce an Kinsella (1978), supra and compared to that of an established protein emulsifier like bovine serum albumin. While there are several methods to evaluate the emulsifying properties, the method used here was based on the determination of the emulsifying activity index which relates the tubidity of the emulsion to the interfacial area of an emulsion and is expressed in m.sup.2 /g. This method may not be completely accurate but it can be effectively used for qualitative comparison of emulsifying activities of different proteins. The results on the emulsifying activity and emulsion stability of the different soy proteins and bovine serum albumin are presently in FIG. 11. Statistical analysis on the emulsifying activity indices reveal a difference ($p<0.05$) among the three soy proteins with the flour exhibiting the highest index. This higher index may be attributed to higher solubility observed in soy flour. Data on the stability of emulsions formed as a function of time reveal that the membrane soy concentrate exhibited the highest index when compared to soy flour and soy isolate. This example suggest that the concentration of the proteins by ultrafiltration does not alter the emulsifying properties of the soy proteins when compared to that of native proteins in soy flour.

Detailed Description Paragraph Right (64):
Based on the chemical scores calculated for the essential amino acid, the membrane soy concentrate when compared to casein is seen to lack marginally (2-5%) in isoleucine, threonine and histidine 14-15% less in leucine and valine with a significant lack in tyrosine (27%) and methionine (50%). This example suggests that the low methionine content of membrane soy concentrate is reflective of the limiting amino acids in soy flour from which the soy concentrate is processed. It seems as if membrane processing does not alter the amino acid pattern of the soy protein after concentration.

Detailed Description Paragraph Right (66):
Using methodology similar to that of Example I, Twenty five (25) pounds of soy protein concentrate was produced. The phytate level of this material was evaluted by the method of McChance and Widdewson, supra. The soy protein concentrate had a phytate level of 0.026 w/w %. By contrast soy flour will typically have a phytate level of 2 to 3 w/w %.

Detailed Description Paragraph Left (3):
Insoluble proteins have very limited use in foods. Nitrogen solubility can be assumed to be reflective of protein solubility. Protein solubility is known to influence functional properties such as foaming, gelation and emulsification. Solubility is influenced by several conditions, pH being an important one. The nitrogen solubility of soy flour, membrane soy concentrate and commercial soy isolate was determined in the pH range between 3.0 and 10.0 by the method outlined by Bera and Mukherjee (1989),

supra and is presented FIG. 10.

Detailed Description Paragraph Table (1):
TABLE 1 Sample Protein Content (%) Soy Flour 51.2 Diafiltered Retentate 56.2 Pectinase Retentate 76.7 Crystalzyme Retentate 78.5 Permeate <0.5

Detailed Description Paragraph Table (3):
TABLE 2 % Sample % Protein % Carbohydrate % Ash Moisture Soy Flour 51.2 15.2 6.2 7.2 Supro 1610 86.7 1.7 4.3 5.2 MSC (Sigma) 76.7 8.9 5.3 5.4 MSC (Crystalzyme) 78.5 5.7 4.9 6.9

Detailed Description Paragraph Table (4):
TABLE 3 Sample Mean Aroma Mean Flavor Soy Flour 56.7.sup.a 50.2.sup.d Supro 1610 53.1.sup.ab 50.8.sup.d MSC (Pectinase) 45.8.sup.b 35.7.sup.e MSC (Crystalzyme) 29.6.sup.c 35.5.sup.e Means with the same letter are not significantly different ($p < 0.05$).

Detailed Description Paragraph Table (9):
TABLE 8 Water Hydration Surface Sample Capacity Hydrophobicity Soy Flour 2.35.sup.a 10.52 Membrane Soy Concentrate 2.61.sup.a 20.65 Supro 1610 5.64 36.77 Means with the same letter are not significantly different ($p < 0.05$).

Other Reference Publication (8):
Rudloff, et al., Calcium and zinc retention from protein hydrolysate formulas in suckling rhesus monkeys, AJDC vol. 146, May 1992.

Other Reference Publication (14):
Baker, et al., Extraction of defatted soybean flours and flakes with aqueous alcohols: Evaluation of flavor and selected properties, Journal of Agricultural and Food Chemistry 1979; 27(5): 969-979.

Other Reference Publication (23):
Kalbrener, et al., Sensory evaluation of commercial soy flours, concentrates and isolates, Cereal Chemistry 1971; 48: 595-600.

Other Reference Publication (25):
Maga, A review of flavor investigations associated with the soy products raw soybeans, defatted flakes and flours, and isolates, Journal of Agricultural and Food Chemistry 1973; 21(5): 864-868.

Other Reference Publication (32):
Sessa, et al., Lipid oxidation in full-fat and defatted soybean flakes as related to soybean flavor, Cereal Chemistry 1969; 46: 675-686.

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DOCUMENT-IDENTIFIER: US 6251443 B1
TITLE: Method for producing a savory flavor base

Abstract Paragraph Left (1):
The present invention relates to a method for producing a flavor base which then can be used to form savory flavor or flavor enhancer products. The method involves enzymatically hydrolyzing cereal grain cereal protein, in particular defatted wheat germ, to produce the flavor base.

Brief Summary Paragraph Right (1):
The present invention relates to a method for producing a savory flavor base, wherein the method includes enzymatically hydrolyzing an amount of cereal protein raw material, most preferably defatted wheat germ, with at least one enzyme. The present invention also relates to methods for forming savory flavor and flavor enhancer compositions from the savory flavor base.

Brief Summary Paragraph Right (3):
HVP is a savory flavor base often used to form savory flavors and flavor enhancers, with the HVP generally described as either a flavor donor, a flavor enhancer, or a combination thereof. The HVP can be formed by hydrolyzing a vegetable protein with an acid or an enzyme. The protein or raw material used to form the HVP typically includes one of the following: wheat gluten, corn gluten, defatted wheat germ, defatted soy flour, defatted peanut flour, and defatted cottonseed flour. Wheat gluten is often the preferred raw material because it has an increased amount of glutamic acid which will allow often for the production of an HVP that has a less bitter, sweeter flavor. The less bitter, sweeter flavor is similar to a yeast extract, meaning the HVP can be used as a replacement for the yeast extract when formulating a savory flavor base. It is desired to use an HVP as opposed to a yeast extract as yeast extracts are comparatively expensive to produce and use. Unfortunately, wheat gluten can be expensive to use as a raw material. Thus, it is desired to have a more economical replacement, such as an HVP, for a yeast extract.

Brief Summary Paragraph Right (4):
Defatted wheat germ when used to form an HVP imparts a desired flavor; however, it also suffers from being expensive to use. It is known that defatted wheat germ contains about 30% by weight peptides or protein. As such, typically the yield of HVP from defatted wheat germ is decreased. Conversely, when defatted soy flour is used to form the HVP, the soy flour typically contains at least 50% by weight protein. Because of the lesser amount of protein found in the defatted wheat germ, a lower yield is realized which in turn makes it less economical to use. Another problem associated with defatted wheat germ is that it is a processed product or an "end" product. This will also increase the cost associated with producing an HVP from defatted wheat germ as the raw material has a higher comparative cost than other raw material. It is desired to produce an HVP from defatted wheat germ that has a sufficient yield and is economical to form.

Brief Summary Paragraph Right (15):
The present invention relates to a method for producing a savory flavor base from a cereal protein, preferably defatted wheat germ, wherein the savory flavor base is formed from enzymatically degraded protein. More particularly, the present invention relates to a method for producing a savory flavor base from cereal grain protein, especially defatted wheat germ, to produce a new and novel savory flavor base having a different taste than what is presently known in other hydrolyzed vegetable protein

(HVP) savory products, while still having characteristics similar to known HVP savory products. It is also preferred if the savory flavor base has a taste that is slightly different than a yeast extract. Most importantly, it should be pointed out that enzymatically hydrolyzing cereal grain protein, especially defatted wheat germ, will produce an HVP or a savory flavor base that has unknown and different flavors from what has been previously produced from other HVP products, especially soy protein products. As such, this is an important new invention because consumers demand healthier, safer, new and different savory flavors and the use of an enzymatically hydrolyzed cereal grain protein, in particular defatted wheat germ, will provide for new and different savory flavor and flavor enhancer products. Also, HVP products produced by enzymatically hydrolyzing defatted wheat germ are novel and are believed to have not been manufactured previously.

Brief Summary Paragraph Right (16):
The present process is initiated by forming a cereal protein slurry by mixing an amount of cereal grain protein with an amount of water. More preferably, the present process is initiated by forming a slurry from an amount of defatted wheat germ and water. Because the defatted wheat germ is preferred, it will be referred to throughout even though potentially other cereal grain proteins could be used in this process. Preferably, the defatted wheat germ slurry is then pasteurized, followed by cooling. To the pasteurized wheat germ slurry a variety of enzymes can be added, with the enzymes added dependent upon the desired flavor characteristics of the flavor base to be produced by the hydrolysis of the defatted wheat germ and dependent upon the pH of the defatted wheat germ slurry. As the enzymes degrade proteins, the pH will be lowered, so that it may be necessary to include enzymes which more readily hydrolyze protein at lower pHs so that as time passes hydrolysis will continue regardless of the pH of the fermenting defatted wheat germ slurry. For this reason, it is desired to use more than one enzyme to produce the savory flavor base. It should be emphasized that the combination of the defatted wheat germ and the enzymes will produce a unique flavor base or HVP that has been previously unknown.

Brief Summary Paragraph Right (17):
After fermentation of the defatted wheat germ slurry, it is preferred to then pasteurize the defatted wheat germ slurry to prevent further enzymatic degradation and to inactivate the enzymes. The defatted wheat germ slurry will then be filtered to separate insoluble materials, and materials which negatively influence the flavor of the savory flavor base, from the soluble protein hydrolysate or flavor base. Upon conclusion of filtering, the savory flavor base can be further concentrated, as well as, having colors, flavors, and other constituents added thereto to form either a savory flavor or a flavor enhancer. Again, this is a new and novel method because it has not been known to enzymatically hydrolyze defatted wheat germ to produce an HVP or savory flavor base. This results in new and novel savory flavors which have not been previously known and which will allow for new and different types of flavor enhanced food products.

Brief Summary Paragraph Right (18):
This method is advantageous because a savory flavor having a new desirable flavor is produced from an HVP or flavor base. The savory flavor will have a less bitter, sweeter flavor, while having strong beef and chicken flavors. Further, the present savory flavor will generally have a lesser amount of salt, meaning it is a healthier savory flavor. The present method is also desirable because it is economical as the raw material, the defatted wheat germ, is occasionally a by-product or is considered a waste product with little economic value. Also, unlike previous enzymatically treated proteins, the yield in the present invention is adequate so that the present invention is inexpensive and has suitable yields from the raw material.

Brief Summary Paragraph Right (19):
The present method relates to the production of a savory flavor base or HVP and a savory flavor and flavor enhancers resulting from enzymatically treating a cereal product, preferably a cereal protein such as defatted wheat germ. The defatted wheat germ will be referred to throughout since it is the raw material and cereal protein of choice. More specifically, the present method relates to the production of a protein hydrolysate or HVP from the defatted wheat germ, with the enzymatically hydrolyzed defatted wheat germ forming a savory flavor base. The hydrolyzed wheat germ can have additional constituents added thereto so that it may be dried to form a savory flavor

or flavor enhancer. The present method involves hydrolyzing the defatted wheat germ by using at least one suitable enzyme in a desired amount.

Brief Summary Paragraph Right (20):
The method is initiated by selecting an amount of defatted wheat germ and mixing such wheat germ with an amount of water to form a wheat germ slurry. The wheat germ can be added to the water in an amount equal to between about 10% and about 20% by weight of said wheat germ slurry, with the amount of wheat germ added dependent upon a variety of factors. The amount of wheat germ added to form the wheat germ slurry will depend upon the particular proteases used, as different proteases break down peptides into amino acids with different efficiencies, the time allowed for fermentation, the particular flavor profile desired, and the amount of protein found in the defatted wheat germ material.

Brief Summary Paragraph Right (21):
The protein of the present method can be selected from the group consisting of cereal grain protein and soy bean derived vegetable protein, including soy grit, soy flakes, soy isolate, soy concentrate, and combinations thereof. The cereal grain protein is most preferred because of the different flavors that can be developed from the cereal grain protein. Among the available cereal grain proteins are defatted wheat germ, pea protein, defatted corn germ, wheat gluten, corn flour, oat meal, peanut protein, and combinations thereof. The most preferred cereal grain protein, as noted, is the defatted wheat germ.

Brief Summary Paragraph Right (22):
In addition to the defatted wheat germ, an amount of salt, preferably sodium chloride (NaCl), can optionally be added to the defatted wheat germ slurry. Generally, the salt will be added in an amount ranging between about 0% and about 10% by weight of the defatted wheat germ slurry and more preferably in an amount ranging between about 5% and about 10% by weight of the defatted wheat germ slurry. The salt is added to secure the microbial stability of the defatted wheat germ slurry for several days of incubation at control temperature conditions. Also, the salt when added in a desired amount will favorably influence the flavor of the savory flavor base.

Brief Summary Paragraph Right (23):
It is preferred to then heat and mix the defatted wheat germ slurry at a sufficient temperature for a sufficient amount of time to prevent bacterial spoilage during enzymatic hydrolysis. As such, preferably the defatted wheat germ slurry is pasteurized or heat treated at a sufficient temperature to kill bacteria, but at a temperature that does not produce a maillard reaction. A desirable pasteurization process includes heating the defatted wheat germ slurry to a temperature of 95.degree. C. for approximately 3 hours.

Brief Summary Paragraph Right (24):
After heating, it is necessary to cool the defatted wheat germ slurry and to adjust the pH so that an enzyme friendly environment is produced. Preferably, the defatted wheat germ slurry is cooled to about 45.degree. C. and the pH is adjusted to approximately 7, however, other environments can be used dependent upon the enzyme or enzymes added to the defatted wheat germ. Thus, the pH will be adjusted dependent upon the enzyme or enzymes used to hydrolyze the defatted wheat germ. Different enzymes will thrive under different pH conditions. The pH can be adjusted using any basic compound that will adjust the pH from a more acidic level to a more neutral level without imparting any undesirable health effects to the defatted wheat germ slurry or unsuitable flavors. An example of a suitable composition for adjusting the pH is sodium hydroxide (NaOH).

Brief Summary Paragraph Right (25):
Once a suitable environment has been established, at least one enzyme, typically a protease, is added to the defatted wheat germ slurry, with the enzyme designed to promote hydrolysis of the defatted wheat germ slurry. Any of a variety of enzymes can be used that will promote hydrolysis of the defatted wheat germ in a slurry having a pH of about 7. The proteases that are selected will be dependent in part upon the desired finished flavor of the HVP or savory flavor base. Different enzymes will break down different peptides giving the flavor base a different flavor profile. Also, different enzymes or proteases will function in different pH conditions so that it is

desired to add different enzymes because as the hydrolysis of the defatted wheat germ occurs, the pH in the slurry will lower and it may be necessary to have enzymes which continue the hydrolysis reaction at a lower pH. Enzymes or proteases useful in the present invention include one or more of any enzyme exhibiting protease activity, including endoproteases and exoproteases. The preferred enzymes include Aspergillus oryzae (FLAVOURZYME.TM.), ALCALASE.RTM., and Aspergillus sp., and VISCOZYME.TM.The ALCALASE.RTM. is a proteolytic enzyme. The FLAVOURZYME.TM. is a protease that functions as both an exoprotease and an endoprotease. The VISCOZYME.TM. is a carbohydrase. Preferably, if all three enzymes are used, then the FLAVOURZYME.TM. will be added in an amount equal to at least 0.3% by weight of the defatted wheat germ slurry, the VISCOZYME.TM. will be added in an amount equal to at least 0.5% by weight of the protein found in the defatted wheat germ slurry, and the ALCALASE.RTM. enzyme will be added in an amount equal to at least 0.5% by weight of the protein found in the defatted wheat germ slurry. Preferably, the FLAVOURZYME.TM. is added in an amount equal to between 3% and 7%, the ALCALASE.RTM. is added in an amount equal to between 0.5% and 1%, and the VISCOZYME.TM. is added in an amount equal to at least 0.5%. Regardless of the particular enzyme, at least one enzyme equal to at least 0.5% by weight of the protein content of the defatted wheat germ is added. Once the enzymes are added, the defatted wheat germ slurry containing the enzymes is allowed to incubate for a sufficient time at a sufficient temperature to promote hydrolysis of the defatted wheat germ slurry. Generally, fermentation or incubation will occur at a temperature ranging between about 45.degree. C. and about 60.degree. C. for a period of time ranging between about 120 and about 165 hours. Preferably, the fermentation step will be carried out at a temperature equal to between about 50.degree. C. and about 60.degree. C. for a period of time ranging between about 5 and about 7 days.

Brief Summary Paragraph Right (26):
After suitable enzymatic hydrolysis has occurred, it is preferred to inactivate the enzymes found in the defatted wheat germ slurry. Generally, this can occur by again pasteurizing or heat treating the slurry. For example, the slurry can be heated to a temperature of about 90.degree. C. for one hour. Another method for inactivating the enzymes can include lowering the pH of the slurry and heating the slurry thereby inactivating the enzymes.

Brief Summary Paragraph Right (27):
Next, the hydrolyzed defatted wheat germ slurry is filtered to separate insoluble material from soluble material, with the soluble material forming a protein hydrolysate. Any of a variety of means can be used to separate the filtrate fraction or protein hydrolysate from the insoluble portion of the defatted wheat germ slurry. Among the available means are membrane filtration and centrifugation. It is preferred, however, to filter the defatted wheat germ material through a membrane filter, such as a plate and frame filter. Regardless of the filter means used it is important to make sure that the carbohydrate and fiber material does not pass through the filter so that the carbohydrate and fiber material is not mixed with the protein hydrolysate. Thus, it is desired to remove insoluble material and unwanted flavor components.

Brief Summary Paragraph Right (28):
It is preferred to then concentrate the soluble protein hydrolysate to a solids level ranging between about 60% and about 65% by weight of the protein hydrolysate. Any of a variety of different means can be used to concentrate the filtrate, however, it is preferred to use either an evaporator or reverse osmosis for evaporation and concentration of the filtrate. The concentrated filtrate is the flavor base or HVP of the present invention. The flavor base will have a unique and desirable flavor not found in yeast extract, HVP formed by acid hydrolysis, or HVP formed from soybean materials.

Detailed Description Paragraph Right (1):
A savory flavor base product was produced by hydrolyzing an amount of defatted wheat germ. The method involved forming a wheat germ slurry by mixing 38 kilograms of water or two (2) parts by weight, with 3.21 grams of NaCl or 8% to 10% by weight of the wheat germ slurry, and 8 kilograms or one (1) part by weight of defatted wheat germ having 30% by weight protein to form the wheat germ slurry. The wheat germ slurry was mixed in a reaction tank having a 100 liter capacity with agitators and being double jacketed for hot and cold water circulation. After formation of the wheat germ slurry, the reaction tank was heated to approximately 95.degree. C. and the wheat germ slurry

was pasteurized for approximately four (4) hours. Upon the conclusion of pasteurization, the pasteurized wheat germ slurry was cooled to approximately 50.degree. C.

Detailed Description Paragraph Right (3):
Following the addition of the NaOH solution, enzymes, or proteases, were added to the pasteurized wheat germ slurry in the reaction tank. The first enzyme added was ALCALASE.RTM. which was added in an amount equal to about 0.5% by weight of the protein content of the defatted wheat germ. As mentioned, the defatted wheat germ had 30% by weight protein so that 12 grams of the ALCALASE.RTM. enzyme was added. Next, 72 grams of a FLAVOURZYME.TM. enzyme was added, with this enzyme added in an amount equal to 3% by weight of the protein content of the defatted wheat germ. Next, 40 grams of a VISCOZYME.TM. enzyme were added, so that the VISCOZYME.TM. was added in an amount equal to 0.5% by weight of the protein found in the defatted wheat germ slurry. All three (3) enzymes were manufactured by NOVO NORDISK BIOCHEM NORTH AMERICA.TM. Franklington, N.C.

Detailed Description Paragraph Right (4):
Fermentation of the defatted wheat germ was then allowed to proceed at 48.degree. C. for approximately five days. At the end of five (5) days 50 kilograms of water were added to the hydrolyzed defatted wheat germ slurry and the mixture was then heated to 90.degree. C. for one (1) hour to inactivate the enzymes so that hydrolysis would discontinue. The amount of water added was equal to the amount of the total slurry. The hydrolyzed protein mixture was then cooled to 50.degree. C. and filtered to separate the insoluble material from the soluble material. The protein hydrolysate slurry was next filtered through a plate and frame filter to remove the insoluble cell wall material.

Detailed Description Paragraph Right (5):
Filtering allowed for the isolation of a soluble protein hydrolysate or flavor base from the hydrolyzed wheat germ slurry. It was observed that the pH of the soluble protein hydrolysate or flavor base was 5.05 after five (5) days.

Detailed Description Paragraph Right (8):
Enzymes were then added to the vegetable protein solution at the following levels: 0.7% ALCALASE.RTM. (44 grams), 3.0% FLAVOURZYME.TM. (264 grams), 0.5% VISCOZYME.TM. (110 grams). The percent of enzyme relates to the amount of protein found in the vegetable protein solution, as explained in Example 1. Hydrolysis was then carried out for five (5) days at 48.degree. C. On day five (5) the mixture was eluted up with an equal amount of water and heated to 90.degree. C. for two (2) hours to inactivate the enzymes. The mixture was then centrifuged using a Westfalia SA-7 diskstack centrifuge to separate the insoluble solid material from soluble protein filtrate. The solids were discarded and the liquid supernatant or protein filtrate was then evaporated to 16-20% by weight solids. The material that was centrifuged yielded 15 liters of sludge or insoluble and 40 liters of protein hydrolysate. The 40 liters of protein hydrolysate contained 11.1% solids. This had an inadequate concentration of solids. Also, the flavor was undesirable.

CLAIMS:

1. A method for producing a savory flavor base, wherein said method consists of:

(a) mixing salt, water, and a defatted wheat germ to form a cereal protein slurry, with said salt added in an amount ranging between about 5% and about 10% by weight of said cereal protein slurry and said protein added in an amount equal to between about 10% and about 20% by weight of said cereal protein slurry;

(b) heating said cereal protein slurry at a temperature and time sufficient to pasteurize said cereal protein slurry;

(c) adding at least one enzyme to said cereal protein slurry;

(d) fermenting said cereal protein slurry for a period of time sufficient to hydrolyze said cereal protein;

(e) inactivating said enzyme to prevent further fermentation; and

(f) filtering said inactivated protein slurry to separate a protein hydrolysate from insoluble protein material.

6. A method for producing a savory flavor base, wherein said method consists of:

(a) forming a defatted wheat germ mixture, with said defatted wheat germ mixture comprised of an amount of defatted wheat germ added in an amount equal to between about 10% and about 20% by weight of said defatted wheat germ mixture;

(b) fermenting said defatted wheat germ mixture with an amount of enzyme for a sufficient amount of time to hydrolyze some of said defatted wheat germ and form a defatted wheat germ hydrolysate; and,

(c) filtering said cereal protein hydrolysate so as to obtain said flavor base.

10. The method of claim 6, wherein said enzyme is added in an amount equal to at least 0.5% by weight of protein in said defatted wheat germ.

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L4: Entry 2 of 7 File: USPT Feb 20, 2001

DOCUMENT-IDENTIFIER: US 6190709 B1
TITLE: Flavor enhancer

Brief Summary Paragraph Right (7):
The present invention provides a flavour enhancer that is low in monosodium glutamate, methods for preparing the flavour enhancer, compositions comprising the flavour enhancer, and uses of the flavour enhancer. A preferred method for preparing the flavour enhancer as a soy protein hydrolysate comprises: (i) forming an aqueous suspension of a soy protein containing starting material (e.g. soy flour, soy protein isolate, soy beans, or soy bean flakes, meal or grits, which preferably are defatted); (ii) heating the aqueous suspension for at least from about 1 minute to about 15 minutes at a temperature of from about 60.degree. C. to about 82.degree. C.; (iii) incubating the suspension with a protease mixture comprising endoprotease and exoprotease activity, to obtain an amino acid level in the suspension of from about 20% to about 55%; (iv) adjusting the pH and temperature of the suspension to inactivate the endoprotease and exoprotease; and (v) recovering the soy protein hydrolysate (e.g. by concentrating and/or drying, or other appropriate means).

Detailed Description Paragraph Right (4):
450 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 2.5 l water at 20.degree. C. in the presence of 0.5% (w/w) Pescalase.RTM. protease (Gist-Brocades, the Netherlands). This suspension was heated for 10 minutes at 75.degree. C. After cooling to 55.degree. C. and adjusting to pH 5.1, the suspension was hydrolysed for 15 hours using 2% (w/w) Sumizyme.RTM. FP protease (Shin Nihon, Japan). After hydrolysis, this mixture was incubated at pH 4.0 and 80.degree. C. for 15 minutes to stop hydrolysis. After cooling to 40.degree. C. the hydrolysate was obtained by centrifugation for 30 minutes at 2200 g. The pellet was washed twice with process water. The resulting slurry was filtered at a pressure of 0.4 to 1 bar using Dicalite 418 as a filter aid. After concentration by rotary evaporation at 40.degree. C., 50 mbar, the filtrate was spray-dried (inlet temperature 130.degree. C., outlet temperature 80.degree. C.). A light coloured powder was obtained.

Detailed Description Paragraph Right (15):
351 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% Pescalase.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry mater of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) Pescalase.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to 5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and 1% (weight/dry weight) Sumizyme.RTM. FP protease (Shin Nihon, Japan) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772.97 on a medium of glucose and Gistex yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water.

Detailed Description Paragraph Right (21):
351 g of defatted soy flour, Nutrisoy.RTM. 7 B flour (53% w/w protein, ADM, The Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% B 500.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry matter of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) B 500.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to 5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and 1% (weight/dry weight) Flavourzyme.RTM. protease (Novo Nordisk A/S, Denmark) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772.97 on a medium of glucose and Gistex.RTM. yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water.

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L4: Entry 3 of 7 File: USPT Dec 28, 1999

DOCUMENT-IDENTIFIER: US 6007851 A
TITLE: Process for producing a flavor enhancer

Brief Summary Paragraph Right (7):
The present invention provides a flavour enhancer that is low in monosodium glutamate, methods for preparing the flavour enhancer, compositions comprising the flavour enhancer, and uses of the flavour enhancer. A preferred method for preparing the flavour enhancer as a soy protein hydrolysate comprises: (i) forming an aqueous suspension of a soy protein containing starting material (e.g. soy flour, soy protein isolate, soy beans, or soy bean flakes, meal or grits, which preferably are defatted); (ii) heating the aqueous suspension for at least from about 1 minute to about 15 minutes at a temperature of from about 60.degree. C. to about 82.degree. C.; (iii) incubating the suspension with a protease mixture comprising endoprotease and exoprotease activity, to obtain an amino acid level in the suspension of from about 20% to about 55% (iv) adjusting the pH and temperature of the suspension to inactivate the endoprotease and exoprotease; and (v) recovering the soy protein hydrolysate (e.g. by concentrating and/or drying, or other appropiate means).

Brief Summary Paragraph Right (11):
The present invention provides, among other things, a soy hydrolysate which is obtainable by: (i) heating a suspension of defatted soy flour in water for at least about 10 min at from about 65.degree. C. to about 82.degree. C.; (ii) incubating the suspension with a mixture of endo- and exo-proteases obtained from Aspergillus species at from about 40.degree. C. to about 60.degree. C. at a pH of about 4 to about 6 for a sufficient time to obtain an amino acid level of 20% to 55%; (iii) lowering the pH to between about 3.5 and about 4.5 and increasing the temperature to from about 80.degree. C. to about 100.degree. C. for a period of time ranging from about 10 minutes to about 4 hours; and (iv) lowering the temperature to from about 25.degree. C. to about 40.degree. C. and, optionally, recovering the hydrolysate.

Detailed Description Paragraph Right (5):
450 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 2.5 l water at 20.degree. C. in the presence of 0.5% (w/w) Pescalase.RTM. protease (Gist-Brocades, the Netherlands). This suspension was heated for 10 minutes at 75.degree. C. After cooling to 55.degree. C. and adjusting to pH 5.1, the suspension was hydrolysed for 15 hours using 2% (w/w) Sumizyme.RTM. FP protease (Shin Nihon, Japan). After hydrolysis, this mixture was incubated at pH 4.0 and 80.degree. C. for 15 minutes to stop hydrolysis. After cooling to 40.degree. C. the hydrolysate was obtained by centrifugation for 30 minutes at 2200 g. The pellet was washed twice with process water. The resulting slurry was filtered at a pressure of 0.4 to 1 bar using Dicalite 418 as a filter aid. After concentration by rotary evaporation at 40.degree. C., 50 mbar, the filtrate was spray-dried (inlet temperature 130.degree. C., outlet temperature 80.degree. C.). A light coloured powder was obtained.

Detailed Description Paragraph Right (14):
351 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% Pescalase.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry matter of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) Pescalase.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to

5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and it (weight/dry weight) Sumizyme.RTM. FP protease (Shin Nihon, Japan) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772.97 on a medium of glucose and Gistex yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water. After a heatshock for 5 min at 95.degree. C., the supernatant was concentrated in a glass evaporator at 60.degree. C. and 120-150 mbar. Afterwards, the pH of the concentrate was adjusted to 5.1 and the material was spray dried.

Detailed Description Paragraph Right (19):
351 g of defatted soy flour, Nutrisoy.RTM. 7B flour (53% w/w protein, ADM, The Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% B 500.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry matter of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) B 500.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to 5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and 1% (weight/dry weight) Flavourzyme.RTM. protease (Novo Nordisk A/S, Denmark) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772. 97 on a medium of glucose and Gistex.RTM. yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water.

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L6: Entry 10 of 26 File: USPT Dec 28, 1999

DOCUMENT-IDENTIFIER: US 6007851 A
TITLE: Process for producing a flavor enhancer

Brief Summary Paragraph Right (7):
The present invention provides a flavour enhancer that is low in monosodium glutamate, methods for preparing the flavour enhancer, compositions comprising the flavour enhancer, and uses of the flavour enhancer. A preferred method for preparing the flavour enhancer as a soy protein hydrolysate comprises: (i) forming an aqueous suspension of a soy protein containing starting material (e.g. soy flour, soy protein isolate, soy beans, or soy bean flakes, meal or grits, which preferably are defatted); (ii) heating the aqueous suspension for at least from about 1 minute to about 15 minutes at a temperature of from about 60.degree. C. to about 82.degree. C.; (iii) incubating the suspension with a protease mixture comprising endoprotease and exoprotease activity, to obtain an amino acid level in the suspension of from about 20% to about 55% (iv) adjusting the pH and temperature of the suspension to inactivate the endoprotease and exoprotease; and (v) recovering the soy protein hydrolysate (e.g. by concentrating and/or drying, or other appropiate means).

Brief Summary Paragraph Right (11):
The present invention provides, among other things, a soy hydrolysate which is obtainable by: (i) heating a suspension of defatted soy flour in water for at least about 10 min at from about 65.degree. C. to about 82.degree. C.; (ii) incubating the suspension with a mixture of endo- and exo-proteases obtained from Aspergillus species at from about 40.degree. C. to about 60.degree. C. at a pH of about 4 to about 6 for a sufficient time to obtain an amino acid level of 20% to 55%; (iii) lowering the pH to between about 3.5 and about 4.5 and increasing the temperature to from about 80.degree. C. to about 100.degree. C. for a period of time ranging from about 10 minutes to about 4 hours; and (iv) lowering the temperature to from about 25.degree. C. to about 40.degree. C. and, optionally, recovering the hydrolysate.

Brief Summary Paragraph Right (12):
The starting material used for hydrolysis may contain from about 50% to about 100% (w/w) soy protein, preferably from about 50% to about 75%. In a preferred embodiment of the invention, defatted non-toasted soy flour (Cargill B. V., the Netherlands) containing about 52% (w/w) soy protein, maximally about 1.5% (w/w) fat, and from about 2 to about 4% (w/w) fibres is used. However, the person skilled in the art will understand that also other defatted soy protein containing material, such as soy protein isolate or toasted soy flour, may be used as starting material. Although soy beans may be used as well, the result can be less satisfactory, due to the oil present in these beans.

Brief Summary Paragraph Right (13):
Advantageously the viscosity may be reduced, e.g. to facilitate further manipulation of the suspension; such reduction in viscosity preferably may be obtained by enzyme addition. Suitable enzyme preparation include Pescalase.RTM. (Gist-brocades, the Netherlands), B500.RTM. (Gist-brocades, the Netherlands) and Viscozyme.RTM. (NOVO Nordisk, Denmark), or enzyme preparations having similar activity. Preferably Pescalase.RTM. protease is used to reduce the viscosity. Although other enzymes, like cellulase e.g. present in Viscozyme.RTM., are known to reduce viscosity, surprisingly we found that a short incubation with a protease, such as Pescalase.RTM. protease, sufficiently reduced the viscosity. For example, about 1 hour incubation at about 60.degree. C. with about 0.5 w/w % Pescalase.RTM. protease sufficiently reduces viscosity of the suspension comprising soy flour.

Brief Summary Paragraph Right (22):
After hydrolysis (optionally combined with fermentation) the pH of the hydrolysed suspension is lowered and the temperature is raised so as to inactivate the enzymes. This will also kill microorganisms which are present, such as Bacillus stearothermophilus (which belongs to the natural microbial flora of untoasted soy flour) or Bacillus coagulans. The pH is preferably adjusted to between about 3.5 and about 4.5, desirably to between about 3.8 and about 4.2. This pH adjustment will result in precipitation of a part of the proteins present in the hydrolysed suspension. The lower the pH, the more protein will precipitate. This precipitated protein will be seperated, e.g., by filtration or centrifugation, from the final soluble soy protein hydrolysate. Although this results in lower production yields for the final product, the final product can now be used in almost any desirable food or feed product, without producing protein precipitate in these food or feed products. E.g. in clear beverages or other products this may be advantageous. Optimally the temperature may be raised to from about 80.degree. C. to about 100.degree. C., for from about 10 minutes to about 4 hours, preferably for from about 10 to about 30 minutes. In a preferred embodiment of the invention, hydrolysis is terminated by incubating for about 15 minutes at a pH of about 4.0 and a temperature of about 80.degree. C.

Brief Summary Paragraph Right (28):
The soy protein hydrolysate is substantially free of 5'-IMP and 5'-GMP, which means that less than about 0.1%. (w/w dry matter) will be present in the soy protein hydrolysate.

Brief Summary Paragraph Right (29):
Preferably less than 0.01% (w/w dry matter) 5'-IMP and 5'-GMP will be present in the soy protein hydrolysate.

Brief Summary Paragraph Right (32):
Although the flavour enhancer according to the invention enhances the richness of the taste of meat-based foodstuffs, the flavour enhancer is particularly suitable for enhancing dairy-type flavour notes (like cheese), vegetable-type notes (e.g. carrot, tomato, mushroom, onion) and spices (e.g. pepper (pepper heat note enhancement), garlic). A particularly new effect of the flavour enhancer according to the invention is the prolonged flavour perception. Addition of the flavour enhancer according to the invention to a food product makes the food product's taste last longer in the mouth (this is called the linger longer.RTM. taste effect). Furthermore creamy-tasting products taste more creamy and will obtain a thicker mouthfeel when the flavour enhancer according to the invention is added to the food product. The use of the soy protein hydrolysate will enhance the creaminess and mouthfeel of the food or feed product, essentially without increasing the viscosity of these food or feed compositions.

Brief Summary Paragraph Left (1):
All percentages based on w/w dry matter. The protein fraction comprises an amino acid level of from about 20% to about 55%. The amount of monosodium glutamate on dry matter is below about 4%, preferably below about 3%, more preferably below about 2.5%. A typical soy protein hydrolysate according to the invention comprises about 1.5% to about 2.5% (w/w dry matter) monosodium glutamate.

Detailed Description Paragraph Right (5):
450 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 2.5 l water at 20.degree. C. in the presence of 0.5% (w/w) Pescalase.RTM. protease (Gist-Brocades, the Netherlands). This suspension was heated for 10 minutes at 75.degree. C. After cooling to 55.degree. C. and adjusting to pH 5.1, the suspension was hydrolysed for 15 hours using 2% (w/w) Sumizyme.RTM. FP protease (Shin Nihon, Japan). After hydrolysis, this mixture was incubated at pH 4.0 and 80.degree. C. for 15 minutes to stop hydrolysis. After cooling to 40.degree. C. the hydrolysate was obtained by centrifugation for 30 minutes at 2200 g. The pellet was washed twice with process water. The resulting slurry was filtered at a pressure of 0.4 to 1 bar using Dicalite 418 as a filter aid. After concentration by rotary evaporation at 40.degree. C., 50 mbar, the filtrate was spray-dried (inlet temperature 130.degree. C., outlet temperature 80.degree. C.). A light coloured powder was

obtained.

Detailed Description Paragraph Right (14):
351 g of defatted soy flour 200/80 (52% w/w protein, Cargill B. V., the Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% Pescalase.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry matter of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) Pescalase.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to 5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and it (weight/dry weight) Sumizyme.RTM. FP protease (Shin Nihon, Japan) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772.97 on a medium of glucose and Gistex yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water. After a heatshock for 5 min at 95.degree. C., the supernatant was concentrated in a glass evaporator at 60.degree. C. and 120-150 mbar. Afterwards, the pH of the concentrate was adjusted to 5.1 and the material was spray dried.

Detailed Description Paragraph Right (19):
351 g of defatted soy flour, Nutrisoy.RTM. 7B flour (53% w/w protein, ADM, The Netherlands) was suspended in 1.5 l water at 60.degree. C. in the presence of 0.5% B 500.RTM. protease (Gist-brocades, the Netherlands). The enzyme was dosed as percentage of the dry matter of the suspension. The temperature of the suspension was raised to 75.degree. C. in 3.5 hours. During cooling of the suspension to 55.degree. C., the pH was raised to 8.0 using KOH. After adding 0.75% (weight/dry weight) B 500.RTM. protease, these conditions were maintained for 2 hours. Then the pH was adjusted to 5.1 using H.sub.2 SO.sub.4, and an inoculum of B. coagulans CBS 772.97 and 1% (weight/dry weight) Flavourzyme.RTM. protease (Novo Nordisk A/S, Denmark) were added to the mixture. The inoculum of B. coagulans CBS 772.97 was made by culturing a frozen culture of B. coagulans CBS 772. 97 on a medium of glucose and Gistex.RTM. yeast extract (Gist-brocades) pH=5 for 16 hours at 55.degree. C. To the suspension about 5.10.sup.3 cells per ml (final concentration of cells in the suspension, after addition of the cells to the suspension) were dosed. The mixture was fermented and hydrolysed at constant pH and temperature for 15 hours. The reaction was terminated by adding H.sub.2 SO.sub.4 to a pH of 4.0 was reached and raising the temperature to 82.degree. C. in 2 hours. After cooling the suspension to 40.degree. C., the non-solubilized material was removed by centrifugation for 30 min at 2200 g. The pellet was washed twice with water.

Detailed Description Paragraph Left (9):
The dough was kneaded, moulded and baked for 6 min. at 270/280.degree. C. The smell of these crackers changed from staled to more fresh, due to the addition of the soy protein hydrolysate. the taste changed from musty, floury to new, roasted, nutty and cheese-like.

Detailed Description Paragraph Table (6):
2000 g flour 1100 g water 100 g yeast 40 g salt 60 g commercial bread improver 800 g fat 10 g soy protein hydrolysate obtained in Example 1

Detailed Description Paragraph Table (7):
984 g flour 330 g water 150 g soft type bakery fat 6 g salt 30 g dextrose 0.070 g cystein 7.5 g Soy protein hydrolysate obtained in Example 1

Other Reference Publication (2):
Baek et al., "Evaluation of the Enzymatic Hydrolysis of defatted soybean meal by response surface methodology," (abstract), Institute of Food Technologists, 1996 IFT

Annual Meeting, p. 120.

CLAIMS:

1. A process for producing a soy protein hydrolysate, the process comprising the steps of:

(i) forming an aqueous suspension of a soy protein containing starting material;

(ii) heating said aqueous suspension for at least from about 1 minute to about 15 minutes at a temperature of from about 60.degree. C. to about 82.degree. C.;

(iii) incubating said suspension with a protease mixture comprising endoprotease and exoprotease activity at from about 40.degree. C. to about 60.degree. C. at a pH of from about 4 to about 6 for a sufficient time to obtain an amino acid level in the suspension of from about 20% to about 55%; and

(iv) adjusting the pH and temperature of said suspension to inactivate said endoprotease and exoprotease and obtain said soy protein hydrolysate,

wherein said hydrolysate: (a) enhances the flavour of meat and a flavour selected from the group, consisting of dairy flavour, vegetable flavour, and spice flavour, (b) enhances the creaminess and mouthfeel of food or feed compositions, or (c) provides for prolonged delivery of taste perception to food or feed compositions.

2. The process according to claim 1, which further comprises recovering said soy protein hydrolysate.

6. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance flavour, wherein said method comprises adding said hydrolysate to food or feed such that the flavour of said food or feed is enhanced.

8. A process for prolonging the taste of a food or feed composition, the process comprising adding a soy protein hydrolysate produced by the process of claim 1 to said food or feed composition.

9. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance the creaminess and mouthfeel of food or feed compositions, without increasing the viscosity of these food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that creaminess and mouthfeel is enhanced.

10. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance the vegetable taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that vegetable taste is enhanced.

11. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance the spicy taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that spicy taste is enhanced.

12. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance the cocoa and/or chocolate taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that cocoa and/or chocolate taste is enhanced.

13. The method of using the soy protein hydrolysate produced by the process of claim 1 to enhance the butter-taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that butter-taste is enhanced.

14. A process for producing a soy protein hydrolysate, the process comprising the steps of:

(i) forming an aqueous suspension of a defatted soy flour;

(ii) heating said aqueous suspension for at least about 5 minutes at a temperature of from about 65.degree. C. to about 82.degree. C.;

(iii) incubating said suspension with an Aspergillus protease mixture comprising endoprotease and exoprotease activity at from about 40.degree. C. to about 60.degree. C. at a pH of from about 4 to about 6 for a sufficient time to obtain an amino acid level in the suspension of from about 20% to about 55%;

(iv) lowering the pH of said aqueous suspension to between about 3.5 and about 4.5 and increasing the temperature to from about 80.degree. C. to about 100.degree. C. for from about 10 minutes to about 4 hours; and

(v) lowering the temperature of said aqueous suspension to from about 25.degree. C. to about 40.degree. C. to obtain said soy protein hydrolysate,

wherein said hydrolysate: (a) enhances the flavour of meat and a flavour selected from the group consisting of dairy flavour, vegetable flavour, and spice flavour, (b) enhances the creaminess and mouthfeel of food or feed compositions, or (c) provides for prolonged delivery of taste perception to food or feed compositions.

15. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance flavour, wherein said method comprises adding said hydrolysate to food or feed such that the flavour of said food or feed is enhanced.

17. A process for prolonging the taste of a food or feed composition, the process comprising adding a soy protein hydrolysate produced by the process of claim 14 to said food or feed composition.

18. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance the creaminess and mouthfeel of food or feed compositions, essentially without increasing the viscosity of these food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that creaminess and mouthfeel is enhanced.

19. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance the vegetable taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that vegetable taste is enhanced.

20. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance the spicy taste of food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that spicy taste is enhanced.

21. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance the cocoa and/or chocolate taste of these food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that cocoa and/or chocolate taste is enhanced.

22. The method of using the soy protein hydrolysate produced by the process of claim 14 to enhance the butter-taste of these food or feed compositions, wherein said method comprises adding said hydrolysate to said food or feed compositions such that butter-taste is enhanced.

23. A process for producing a soy protein hydrolysate, the process comprising the steps of:

(i) forming an aqueous suspension of a soy protein containing starting material;

(ii) heating said aqueous suspension for at least from about 1 minute to about 15 minutes at a temperature of from about 60.degree. C. to about 82.degree. C.;

(iii) incubating said suspension with a protease mixture comprising endoprotease and

exoprotease activity at from about 40.degree. C. to about 60.degree. C. at a pH of from about 4 to about 6 for a sufficient time to obtain an amino acid level in the suspension of from about 20% to about 55%; and

(iv) adjusting the pH and temperature of said suspension to inactivate said endoprotease and exoprotease and obtain said soy protein hydrolysate,

wherein said hydrolysate is a flavour enhancer.

24. A process for producing a soy protein hydrolysate, the process comprising the steps of:

(i) forming an aqueous suspension of a defatted soy flour;

(ii) heating said aqueous suspension for at least about 5 minutes at a temperature of from about 65.degree. C. to about 82.degree. C.;

(iii) incubating said suspension with an Aspegillus protease mixture comprising endoprotease and exoprotease activity at from about 40.degree. C. to about 60.degree. C. at a pH of from about 4 to about 6 for a sufficient time to obtain an amino acid level in the suspension of from about 20% to about 55%;

(iv) lowering the pH of said aqueous suspension to between about 3.5 and about 4.5 and increasing the temperature to from about 80.degree. C. to about 100.degree. C. for from about 10 minutes to about 4 hours; and

(v) lowering the temperature of said aqueous suspension to from about 25.degree. C. to about 40.degree. C. to obtain said soy protein hydrolysate,

wherein said hydrolysate is a flavour enhancer.

L4: Entry 4 of 7 File: USPT Dec 7, 1999

DOCUMENT-IDENTIFIER: US 5998190 A
TITLE: Enzyme with protease activity

Detailed Description Paragraph Right (61):
Soy flour (prepared from defatted and peeled soy beans) were pelletized at 95.degree. C. and grinded afterwards. The soy flour is suspended in deionized water to 15% dry substance. 5 mg protease I enzyme protein per g of dry substance and 5 mg protease II enzyme protein per g of dry substance, respectively, was added to the soy slurry. The slurry was incubated at 40.degree. C. and pH 5-6. The viscosity in the slurry was measured after 1, 2 and 24 hours of incubation on a Brookfield LV DV III viscometer using a small sample adaptor with spindle #31 at 250 rpm. The residual viscosities were as follows:

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L6: Entry 11 of 26 File: USPT Dec 7, 1999

DOCUMENT-IDENTIFIER: US 5998190 A
TITLE: Enzyme with protease activity

Brief Summary Paragraph Right (5):
Acid proteases are widely used industrially, e.g. in the preparation of food and feed, in the leather industry (e.g. to dehair hides), in the production of protein hydrolysates and in the wine making and brewing industry.

Brief Summary Paragraph Right (55):
Further the enzyme or enzyme preparation of the invention may be useful to make protein hydrolysates from, e.g., vegetable proteins like soy, pea, lupin or rape seed protein, milk like casein, meat proteins, or fish proteins. The protease may be used for protein hydrolysates to improve the solubility, consistency, taste, or fermentability, to reduce antigenicity or for other purposes to make food, feed or dedical products. The protease may be used alone or together with other proteases or together with other enzymes like exopeptidases. The use of the protease of the invention together with exopeptidase rich enzyme preparations will improve the taste of the protein hydrolysates.

Detailed Description Paragraph Right (61):
Soy flour (prepared from defatted and peeled soy beans) were pelletized at 95.degree. C. and grinded afterwards. The soy flour is suspended in deionized water to 15% dry substance. 5 mg protease I enzyme protein per g of dry substance and 5 mg protease II enzyme protein per g of dry substance, respectively, was added to the soy slurry. The slurry was incubated at 40.degree. C. and pH 5-6. The viscosity in the slurry was measured after 1, 2 and 24 hours of incubation on a Brookfield LV DV III viscometer using a small sample adaptor with spindle #31 at 250 rpm. The residual viscosities were as follows:

Detailed Description Paragraph Right (82):
Ground defatted feed quality soy was mixed with deionised water under the conditions described below. The hydrolysis was carried out in two steps in order to simulate the pH conditions in the stomach and the small intestine. The performance of Protease II of the invention was compared with that of Bio-Feed Pro, which by Brenes et al., 1993, has been demonstrated to result in improved weight gain and feed efficiency when used in broiler diets.

Detailed Description Paragraph Table (3):
Hydrolysis conditions:
Hydrolysis mixture 70 g ground defatted soy 330 g deionised water Temperature 40.degree. C. pH 1st step 4.0 2nd step 6.5 Time 1st step 180 minutes 2nd step 180 minutes Enzymes 1st step I) Pepsin 1.92 g (Merck art. 7190) II) I + Bio-Feed Pro 3.0 L 5.5 AU/kg soya III) I + protease II 0.19 AU/kg soya 2nd step I) Pancreatin 6 g (Sigma P 1750) II) I + Bio-Feed Pro 3.0 L 5.5 AU/kg soya III) I + Protease II 0.19 AU/kg soya

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L6: Entry 20 of 26 File: USPT Dec 31, 1991

DOCUMENT-IDENTIFIER: US 5077062 A
TITLE: Hydrolyzed soy protein and process for preparing soy protein

Abstract Paragraph Left (1):
A low sodium, low monosodium glutamate soy hydrolysate is prepared from a soy material, as for instance, soy flour, soy meal or soy grits by hydrolyzing the soy material with a protease enzyme in water. The hydrolysis is conducted in the absence of the addition of either acid or base at a temperature of about 90.degree. for 2 hours. After deactivating the enzyme and dewatering the mixture the resulting hydrolysate contains from about 45 to about 55 weight percent of enzymatic hydrolyzed soy based protein, from about 1 to about 3 weight percent fat, from about 5 to about 9 weight percent ash, from about 2 to about 8 weight percent water, from about 32 to about 36 weight percent carbohydrate, and less than 0.1 weight percent sodium.

Brief Summary Paragraph Right (11):
The objects of the invention are also achieved in a process for preparing such a hydrolyzed soy protein which comprises selecting a soy material from the group consisting of soy flour, soy meal and soy grits and admixing this material with a quantity of water. A quantity of a protease enzyme is added to the soy product in the water. The mixture of the soy material and the enzyme in the water is then mixed while heating to a temperature of from about 85.degree. F. to about 100.degree. F. The mixture is maintained at this temperature for a time period of from about 1 hour 45 minutes to about 2 hours 15 minutes to partially enzymatically hydrolyze the protein in the soy material. The enzyme is then deactivated and the mixture is dried to a solids level of about 90% solids.

Brief Summary Paragraph Right (18):
Typically the enzymatically hydrolyzed soy protein in the product of the invention has an average molecular weight of about 670,000 based on a standard molecular weight of about 1,400,000 for an unhydrolyzed soy flour protein. The soy hydrolysate of the invention, as isolated using the process of the invention, typically also has a metals content equivalent to that of the starting soy material insofar as no additional metals are added because of acid or base hydrolyzing conditions. Potassium is thus present at a level less than about 2.5 weight percent, calcium, magnesium and phosphorus are present at a level of less than 0.1 weight percent and other metals including aluminum, barium, chromium, copper, iron, manganese, strontium and zinc are present at amounts less than 0.01 weight percent.

Drawing Description Paragraph Right (3):
FIG. 2 is a graph of the molecular weight distribution of a soy flour utilized as the starting material in the process of the invention for making a soy hydrolysate of the invention.

Detailed Description Paragraph Right (2):
Soy beans are grown in large quantities in both the United States and in other countries. After harvesting, the soy beans can be stored or processed directly. For processing, they are first screened to remove foreign material and then cracked. The hulls are removed from the cracked beans leaving bean chips. These bean chips are conditioned by heating and then passed through flaking rollers to yield full fat flakes. The full fat flakes are treated with solvents (generally hexane) in a solvent extraction tower. The soy bean oil as well as certain other lipid components, i.e. lecithin, are soluble or miscible in the solvent and are separated from the defatted flakes. Soy bean oil and lecithin phospholipids can be recovered from the solvent by

distillation or other similar processes.

Detailed Description Paragraph Right (3):
The defatted flakes are treated to remove excess solvent and then cooked and toasted. The product is then cooled and ground to a meal or to grits. These in turn can be ground further to flour. The grits, meal or flour generally contains from about 45 to about 60% protein depending upon subsequent processing or additives added thereto. Alternatively, after cooking and toasting the defatted flakes can be treated to remove the sugars to yield a higher percentage protein product, i.e. a product having about 65% protein. Even higher protein products approaching 90% protein can be obtained by isolating the protein from all other components of the defatted flake.

Detailed Description Paragraph Right (4):
The lecithin removed from the defatted flakes by the solvent extraction can be separated from the oil and added back to the defatted flakes to increase the fat content of the soy flour or soy grits.

Detailed Description Paragraph Right (5):
Typically, to produce a soy based hydrolyzed vegetable protein via the prior art processes, a deoiled soy material, i.e. as for instance soy flour, soy grits, soy meal, is acid hydrolyzed. The acid used in the acid hydrolysis is then neutralized and a dry product obtained by spray drying or the like. Typically, this soy based hydrolyzed vegetable protein contains up to 45 or 50% salts and around 10% monosodium glutamate. This product generally has a pH of about pH 5.2 or 5.3. When judged strictly on its flavoring characteristics, such hydrolyzed vegetable protein is extremely useful, however, when judged from an overall total health standpoint, the upwards of 50% salt content and the 10% monosodium glutamate content of this hydrolyzed protein detracts from its overall characteristics and acceptability for use in food products directed to those who are more health conscious.

Detailed Description Paragraph Right (9):
The soy hydrolysate of the invention and the process for preparing the same utilizes a deoiled soy material, as for instance soy flour, soy meal or soy grits, as the basic starting material. This material is suspended in water and a quantity of a protease enzyme added thereto. The suspension is heated to a processing temperature to solubilize both the material and the enzyme and held at this processing temperature for a period of time sufficient to hydrolyze a particular percentage of the peptide bonds of the protein within the soy material. Since no additional acid or base is added during the hydrolysis, the hydrolysis is conducted at essentially the pH of the material itself. Such pH generally ranging from about 6.6 to about 7.2.

Detailed Description Paragraph Right (11):
After hydrolysis, utilizing the essentially neutral pH enzymatic hydrolysis conditions of the invention, the degree of hydrolysis of the resulting sodium hydrolysate is from about 50 to about 53% with the typical product having about 53% of the peptide bonds undergoing hydrolysis. The molecular weight of this product is about 670,000 as compared to a molecular weight of about 1,400,000 for a standard soy flour which could serve as a starting material for the hydrolysis reaction of the invention.

Detailed Description Paragraph Right (13):
The plots of FIGS. 1 and 2 show a size exclusion chromatography plot a soy hydrolysate of the invention in FIG. 1 and of a standard soy flour which has not been hydrolyzed in FIG. 2. As is evident from comparing the plots of FIG. 1 and FIG. 2, the plot of FIG. 1 shows considerably increase of longer retention time fractions indicating a decrease in the molecular size of the sample of the plot of FIG. 1 compared to that of FIG. 2.

Detailed Description Paragraph Right (14):
In addition to the chromatographic plots other information can be extracted from such size exclusion chromatography as is shown in Tables 1 and 2 below. Table 1 is a molecular weight distribution report for a soy hydrolysate of the invention and Table 2 a similar molecular weight distribution report for the soy flour reference standard sample of FIG. 2.

Detailed Description Paragraph Right (15):

In addition to protein, the soy hydrolysate of the invention as well as the referenced soy flour of FIG. 2, contain other molecular moieties including a carbohydrate fraction and lipid fraction. These fractions also show up in the plots of the size exclusion chromatography and would be essentially unchanged by the hydrolysis reaction of the invention. For Tables 1 and 2, the polydispersivity quantitatively measures the breadth of molecular weight distribution with a value 1 being obtained for a completely monodispersed sample. The number average molecular weight (Mm) is an index of the smaller molecules in the distribution. The weighted average molecular weight (Mw) is an index of slightly larger molecules, and the Z average molecular weight (Mz) refers to the high molecular weight components of the distribution.

Detailed Description Paragraph Right (16):
In a strictly qualitative manner it can be considered that the soy hydrolysate of the invention has a molecular weight of about 670,000.+-.50,000 based on a standard molecular weight of about 1,400,000.+-.50,000 for an unhydrolyzed soy flour protein. This represents a degree of hydrolysis of about 53%. As is evident from the plot of FIG. 1, there is little increase in the proportion of sample which is retained beyond about 16 minutes indicating that the soy hydrolysate of the invention is not hydrolyzed to such an extent that an overly abundant amount of free amino acids ar liberated. The hydrolysis is a limited hydrolysis--not a complete hydrolysis to free amino acids. Thus, the hydrolysis of the invention results in the formation of oligopeptides and not free amino acids. This is of particular importance with respect to glutamic acid. Glutamic acid comprises about 20% of the amino acids of soy protein. By avoiding the formation of glutamic acid, the process of the invention avoids the formation of monosodium glutamate.

Detailed Description Paragraph Right (18):
1800 pounds of soy flour was admixed with 4500 pounds of water in a blender equipped with a high shear solubilizing impeller. 1500 grams of a fungal protease enzyme, i.e. Milezyme AFP 2000 available from Miles Laboratories, Elkhart, Ind., was added. At this point the solids, i.e. the soy flour and the enzyme, represents a 28% weight percent suspension of solids in the water. The soy flour and the protease enzyme in the water was stirred and steam was injected into the open reaction vessel. Steam was injected until a temperature of 90.degree. F. was achieved. Steam and water contained therein to achieve this temperature resulted in an addition of 630 additional pounds of water thus diluting the solids content down to 26% weight percent solids. The resulting thick solution of soy flour and enzyme in water was stirred at 90.degree. F. for 2 hours. The solution was then heated to 185.degree. by steam injection to heat kill the enzyme. The resulting solution was then spray dried to yield a soy hydrolysate product of the invention. The product was dried to achieve a moisture content of below 20%, but preferable below 8%. The final moisture of the product ranged from 98% solids to about 92% solids based on a weight percentage.

Detailed Description Paragraph Right (19):
The enzyme is used at a level of about 1000 to about 2000 grams per 1800 pounds soy flour. The percent of the solids, i.e. the soy flour and the enzyme to water, typically ranges from 18 weight percent solids to about 30 weight percent solids with a range of 26 to 28 percent being preferred. During enzymatic hydrolysis the temperature is maintained between 85.degree. and 95.degree. F. with a temperature of about 90.degree. F. preferred. The hydrolysis is conducted from about 1 hour 45 minutes to about 2 hours 15 minutes with a time period of about 2 hours preferred. As noted above, the final product is dried to achieve a preferred moisture level of about 2 to about 8%, however, moistures levels up to about 20% might be acceptable depending upon the final use of the product.

Detailed Description Paragraph Right (21):
Useful for starting materials for the soy hydrolysate of the invention are soy flour, soy meal and soy grits with soy flour being preferred because of its particle size and its ease of solubility.

Detailed Description Paragraph Right (32):
It is, of course recognized that the amino acid content will be somewhat variable depending upon the protein content of the soy product, i.e. the soy flour, the soy meal or the soy grits, utilized as a starting material. For this reason the amino acid values are given a variance of .+-.0.5% for those amino acids which are present at

less than 10% of the total protein and .+-.1.0% for those amino acids which are present in an amount greater than 10% of the total protein.

Detailed Description Paragraph Right (34):
Typically, potassium is present at a level of about 2.0 to 2.5 in the soy flour, soy meal or soy grit starting material used in the process of the invention. As such, the potassium level of the final soy hydrolysate of the invention is at a level less than about 2.5 weight percent. Calcium, magnesium and phosphorus are present in amounts below about 0.1 weight percent with other metals only present in amounts less than 100 parts per million or 0.01 weight percent. Such other metals include aluminum, barium, chromium, copper, iron, magnesium, strontium and zinc. These metals may be completely absent if the soy flour, soy mill or soy grit utilized for the starting material for the process of the invention does not contain such metals, however, if they are, in fact, contained in the soy flour, soy grit or soy meal, the metals will be present in the final product at the same level as they were in the starting material.

Detailed Description Paragraph Right (35):
It is evident that the soy hydrolysate of the invention maintains the same nutritional values as soy protein from raw soy beans since the individual amino acids of the protein are maintained and since physiologically necessary minerals, i.e. calcium, phosphorus, iron, etc., are also maintained in the soy hydrolysate produced from the soy flour, soy meal or soy grits.

Detailed Description Paragraph Table (2):
TABLE 2 _____ SOY FLOUR REFERENCE _____ MOLECULAR WEIGHT DISTRIBUTION.sup.1 Number Average (Mn) 193240 Weight Average (Mw) 1430180 Z Average (Mz) 3619014 Polydispersity 7.401 ESTIMATED % HYDROLYSIS Sample Mw: 1430180 Standard Mw: 1430180 Estimated % Hydrolysis: 0.0 _____ .sup.1 SEPARON HEMABIO 300 SEC Column, 8 .times. 250 mm, A 280 nm. Mobile phase: 0.2M Na2CO3, 1 ml/min.

CLAIMS:

11. A soy protein hydrolysate comprising:

a protein containing soy material selected from the group consisting of soy flour, soy meal and soy grits enzymatically hydrolyzed such that from about 50 to about 55% of the peptide bonds of the protein of said soy material are hydrolyzed and said protein has an amino acid composition of about 4.4.+-.0.5% isoleucine, 8.0.+-.0.5% leucine, 6.4.+-.0.5% lysine, 5.1.+-.0.5% phenylalanine, 4.3.+-.0.5% threonine, 1.0.+-.0.5% tryptophan, 4.6.+-.0.5% valine, 1.3.+-.0.5% methionine, 1.2.+-.0.5% cystine, 4.6.+-.0.5% alanine, 7.1.+-.0.5% arginine, 12.1.+-.1.0% aspartic acid, 19.8.+-.1.0% glutamic acid, 4.4.+-.0.5% glycine, 2.6.+-.0.5% histidine, 5.7.+-.0.5% proline, 5.8.+-.0.5% serine, and 3.4.+-.0.5% tyrosine;

less than 0.1 weight percent of sodium; and

less than 0.1 weight percent of monosodium glutamate.

12. A soy protein hydrolysate of claim 11 including:

said hydrolysate having a pH of from about 6.6 to about 7.2.

13. A soy protein hydrolysate of claim 11 wherein:

said enzymatically hydrolyzed protein of said soy material has an average molecular weight of about 670,000.+-.50,000 based on a standard molecular weight of about 1,400,000.+-.50,000 for an unhydrolyzed soy flour protein.

14. A soy protein hydrolysate of claim 11 further including:

metals individually chosen from the group consisting of calcium, magnesium and phosphorous present in an amounts less than about 0.1 weight percent;

potassium present at a level less than about 2.5 weight percent; and

metals individually chosen from the group consisting of aluminum, barium, chromium, copper, iron, manganese, strontium and zinc present in amounts not exceeding 0.01 weight percent.

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L4: Entry 5 of 7 File: USPT Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5854050 A
TITLE: Enzyme with protease activity

Detailed Description Paragraph Right (83):
Soy flour (prepared from defatted and peeled soy beans) were pelletized at 95.degree. C. and grinded afterwards. The soy flour is suspended in deionized water to 15% dry substance. 5 mg protease I enzyme protein per g of dry substance and 5 mg protease II enzyme protein per g of dry substance, respectively, was added to the soy slurry. The slurry was incubated at 40.degree. C. and pH 5-6. The viscosity in the slurry was measured after 1, 2 and 24 hours of incubation on a Brookfield LV DV III viscometer using a small sample adaptor with spindle #31 at 250 rpm. The residual viscosities were as follows:

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L6: Entry 12 of 26 File: USPT Dec 29, 1998

DOCUMENT-IDENTIFIER: US 5854050 A
TITLE: Enzyme with protease activity

Brief Summary Paragraph Right (6):
Acid proteases are widely used industrially, e.g. in the preparation of food and feed, in the leather industry (e.g. to dehair hides), in the production of protein hydrolysates and in the wine making and brewing industry.

Brief Summary Paragraph Right (56):
Further the enzyme or enzyme preparation of the invention may be useful to make protein hydrolysates from, e.g., vegetable proteins like soy, pea, lupin or rape seed protein, milk like casein, meat proteins, or fish proteins. The protease may be used for protein hydrolysates to improve the solubility, consistency, taste, or fermentability, to reduce antigenicity or for other purposes to make food, feed or dedical products. The protease may be used alone or together with other proteases or together with other enzymes like exopeptidases. The use of the protease of the invention together with exopeptidase rich enzyme preparations will improve the taste of the protein hydrolysates.

Detailed Description Paragraph Right (83):
Soy flour (prepared from defatted and peeled soy beans) were pelletized at 95.degree. C. and grinded afterwards. The soy flour is suspended in deionized water to 15% dry substance. 5 mg protease I enzyme protein per g of dry substance and 5 mg protease II enzyme protein per g of dry substance, respectively, was added to the soy slurry. The slurry was incubated at 40.degree. C. and pH 5-6. The viscosity in the slurry was measured after 1, 2 and 24 hours of incubation on a Brookfield LV DV III viscometer using a small sample adaptor with spindle #31 at 250 rpm. The residual viscosities were as follows:

Detailed Description Paragraph Right (109):
Ground defatted feed quality soy was mixed with deionised water under the conditions described below. The hydrolysis was carried out in two steps in order to simulate the pH conditions in the stomach and the small intestine. The performance of Protease II of the invention was compared with that of Bio-Feed Pro, which by Brenes et al., 1993, has been demonstrated to result in improved weight gain and feed efficiency when used in broiler diets.

Detailed Description Paragraph Table (3):
Hydrolysis conditions:
Hydrolysis mixture 70 g ground defatted soy 330 g deionised water Temperature 40.degree. C. pH 1st step 4.0 2nd step 6.5 Time 1st step 180 minutes 2nd step 180 minutes Enzymes 1st step I) Pepsin 1.92 g (Merck art. 7190) II) I + Bio-Feed Pro 3.0 L 5.5 AU/kg soya III) I + protease II 0.19 AU/kg soya 2nd step I) Pancreatin 6 g (Sigma P 1750) II) I + Bio-Feed Pro 3.0 L 5.5 AU/kg soya III) I + Protease II 0.19 AU/kg soya

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L6: Entry 22 of 26 File: USPT Nov 20, 1984

DOCUMENT-IDENTIFIER: US 4483874 A
TITLE: Preparation of milk substitute

Abstract Paragraph Left (1):
Preparation of a proteinaceous material suited for use in milk substitutes by treatment of crude vegetable proteins such as soy flour, faba bean flour and the like with an SPS-ase preparation. In addition to conversion of the vegetable protein, the treatment hydrolyzes the soluble polysaccharide content in the crude vegetable protein into predominantly mono- and di-saccharides, including dissolving +hydrolyzing previously insoluble polysaccharides.

Brief Summary Paragraph Right (5):
By practice of this invention, inexpensive forms of vegetable protein may be converted into a water soluble protein and carbohydrate product suited to preparation of a milk substitute therefrom. The product is soluble or dispersable at pH 4.5. As the art well knows, vegetable proteins are ill-adapted for use in milk substitutes, (see, for example, the discussion posed in U.S. Pat. No. 3,843,828), and must be converted into a proteinaceous substance better adapted to milk substitute purposes. The purity of the vegetable protein to be converted has received attention by the art, as witness the oft repeated comment in U.S. Pat. No. 3,843,828, that an isolated vegetable protein should be employed. The patent describes isolation of protein from soy flakes. Isolation of the protein is required, because the carbohydrate component present in vegetable protein forms such as soy flour, cottonseed meal, faba bean flour, etc. available inexpensively and almost directly from vegetable oil extraction facilities are not considered desirable for inclusion in milk substitutes. For lack of more apt terminology, soy flour and the like are herein termed relatively crude protein forms, to distinguish such forms from the protein isolates recoverable therefrom, containing less polysaccharides per unit weight than the crude form.

Brief Summary Paragraph Right (7):
Practice of this invention involves concurrent conversion of both protein and polysaccharide in the relatively crude vegetable proteins such as soy flour, faba bean flour, cottonseed meal and the like to form directly products suited for employment as a step-product in production of a milk substitute. The products may be marketed as such in concentrate form for others to employ in production of milk substitutes. Other uses for the products also exist. Soy milk is employed for preparation of tofu, and an improved soy milk can be prepared by practice of this invention.

Brief Summary Paragraph Right (9):
Briefly stated, this invention comprises treating a relatively crude vegetable protein such as soy flour or fullfat soy flour, faba bean flour and the like, in suspension at pH 4-5, preferably at pH 4.5 with an SPS-ase preparation, preferably the SPS-ase of Aspergillus aculeatus CBS 101.43. CBS is Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. With this enzyme it is possible to obtain high product yields and quality products. SPS-ase and the SPS-ase preparations herein preferred are described in detail by U.S. patent application, Ser. No. 334,329, reference thereto being made for full description of this enzyme, and its properties and its preparation from the microorganism source thereof.

Brief Summary Paragraph Right (10):
The usual vegetable protein in relatively crude form available commercially e.g., defatted soy flour, faba bean flour, deoiled cotton seed meal, etc. contain proteins that are not water soluble at their isoelectric points. Practice of this invention

converts the vegetable protein into lower molecular weight proteinaceous substances that are more soluble at the isoelectric point of the vegetable protein, and in particular are largely water soluble at pH 4.5.

Brief Summary Paragraph Right (11):
Practice of this invention is characterized also by capability for converting polysaccharides into the desirable mono- and di-saccharides. A substantial polysaccharide content is present in crude vegetable protein forms such as soy flour, faba bean flour, deoiled cottonseeds, etc. Water insoluble polysaccharides would, of course, be removed by separation following enzymatic conversion of the vegetable protein into a more water soluble proteinaceous substance. However, not all polysaccharides in vegetable protein forms are water insoluble. Absent presence of appropriate carbohydrase activity during the enzymatic digestion, a significant water soluble polysaccharide content accompanies the protein through all dissolution and precipitation steps in the conversion thereof, to appear finally in the milk substitute as an undesired polysaccharide component. Care must be taken so that carbohydrates accompanying the protein are not high in oligosaccharides, since oligosaccharides are known to be responsible for diarrhea and flatulence when given to calves in quantity. It is believed by the inventor hereof that fear of oligosaccharides underlies suggestions to employ vegetable protein isolates for preparation of milk substitute.

Brief Summary Paragraph Right (13):
Preferred relatively crude proteins for practice of this invention are soy flour and faba bean flour.

Brief Summary Paragraph Right (14):
Comtemplated for practice of this invention is employment of fullfat soy flour, milled dehulled cotton seeds and the like in whole or in part for the protein source. The oil content therein would become part of the conversion product, requiring then less extraneous fat for forming a milk substitute.

Brief Summary Paragraph Right (16):
The SPS-ase, hydrolyzes SPS, which substance is a water soluble polysaccharide present in crude soy protein and in the protein isolate as well, and it seems in other comparable vegetable source proteins. The SPS-ase preparations contain carbohydrase activites besides SPS-ase as such, notably pectinase, cellulase, and hemicellulase activities. Hydrolysis of SPS is believed to require more than one carbohydrase activity. In addition, a significant proteinase activity is normally present in the SPS-ase preparations. For practice of this invention, the SPS-ase preparation is an all purpose enzyme. It alone can be employed for conversion of soy flour into a proteinaceous substance suited for milk but supplementation may be desirable. If the relatively crude vegetable protein contains a substantial starch content as does faba beans or field peas for example, starch liquefaction by alpha-amylase before, after or along with treatment by SPS-ase is desirable. Addition of some proteinase may sometimes be needed.

Brief Summary Paragraph Right (19):
In contradistinction to such a practice, the rationale of the present invention is to secure a vegetable protein hydrolysate which is at least largely water soluble balance dispersible or entirely water soluble at the original isoelectric point. For practice of this invention interest is primarily in water solubility and dispersibility in the range of pH 4-5 rather than at the isoelectric point as such. Thus treatment of the crude soy protein with the SPS-ase preparation is at pH 4.0-5.0, preferably at about pH 4.5.

Brief Summary Paragraph Right (22):
Traditionally, soy milk is produced by soaking soy beans in boiling water, wet milling, then extracting with hot water, followed by separation. The liquid phase from the separation is the soy milk. According to practice of this invention, the soy milk is produced by liquefaction of milled soy beans or of soy flour defatted or fullfat by SPS-ase followed by homogenization of the resulting mixture. The soaking step may be eliminated, and/or other enzymes may be included. The optimal reaction parameters for the enzyme reaction for each combination of enzyme and raw material used may be established by cut and try methods, including for example a scan of dosage levels for

the particular substrate and enzyme batch with of temperature and reaction time. The temperature should be 25.degree. C.-50.degree. C., preferably at the upper end of this range. It is vital (to obtain the wanted product) that the temperature chosen be sufficiently low to avoid distruction of essential activities in the SPS-ase preparation. Thus the temperature should not exceed 50.degree. C., and somewhat lower operating temperature levels may be required.

Brief Summary Paragraph Right (29):
Reference is now made to the attached drawing flowsheet showing the conversion of soy flour into a milk substitute ingredient. A final formulation addition of lard, minerals, whey, etc. may be made before final drying or before pasteurization. The SPS-ase treated soy flour is an emulsifier which spontaneously emulsify any fat added in liquid form or melted form. Use of pressure homogenizers are not necessary. A rapid stirrer is enough. For further understanding of this invention, the following Examples of practice thereof are presented. The detailed characteristics of KRF 68, the SPS-ase employed in the examples are provided in S.N. 334,329.

Brief summary Paragraph Center (1):
This invention relates to the preparation of milk substitutes, and in particular, to conversion of a vegetable source proteinaceous material such as soy flour or a fullfat soy flour and the like into a protein and carbohydrate step-product capable of emulsifying fats or oils so as to form a milk substitute. The modified protein is largely or entirely water soluble at pH 4.5. The polysaccharides present in the vegetable source proteinaceous material are hydrolyzed with substantial production therefrom of mono- and di-saccharides. Both the step-products and milk substitutes made therewith are believed to be novel.

Detailed Description Paragraph Right (3):
Soy flour (Sojamel 13) was jet cooked at 150.degree. C. for 25 seconds as described in Example 8 in Ser. No. 334,330 filed Dec. 24, 1981. The jet cooked soy flour was spray-dried and used for the studies described in the following.

Detailed Description Paragraph Right (4):
50 g of the jet cooked soy flour was mixed with 450 g of water, and pH was adjusted to 4.5 with 4.1 ml 6N HCl. The mixture was then heated to 45.degree. C. in a water bath, and 0.250 g of the SPS-ase preparation KRF-68 was added to the heated mixture which was then reacted for five (5) hours with stirring. Thereafter, the mixture was heat treated at 80.degree. C. for two (2) minutes in order to inactivate the enzyme. For analysis of the results a 100 ml sample was centrifuged at ambient temperature for (15) minutes at 3000.times.g (g=gravity). The supernatant was ion-exchanged and analyzed for carbohydrate composition by HPLC. Also, the supernatant was analyzed for Kjeldahl-N and dry matter and the nitrogen solubility index (NSI) and the dry matter solubility index (DSI) was calculated; vide results in Table I. 100 ml of the reaction mixture cooled to 20.degree. C. was poured in a 100 ml graduated glass and kept at 4.degree. C. for two (2) days. The dispersion stability (%) was measured by reading the volume of the dispersions obtained (Table II) after one (1) and two (2) days.

Detailed Description Paragraph Right (11):
In each of 5 flask reactors with stirrer and temperature control 100 g of jet cooked soy flour (see example 2) was mixed with 900 g of water. pH was adjusted to 4.5 by means of 5 ml 6N HCl. SPS-ase (KRF-68) was added in the following dosage:

Detailed Description Paragraph Table (3):
Substrate: Full fat soy flour (Dansk Sojakagefabrik A/S) Mass of Reaction mixture: 220 g Mass of substrate: 20 g Temperature: 50.degree. C. pH: 4.5 (6 N HCl) Reaction Time: Series A: 1 hour Series B: 0.5-6 hours Enzyme: SPS-ase (KRF:68) Enzyme dosage: Series A: E/S-ratio (w/w): 0-8.0% Series B: E/S-ratio (w/w): 1.0%

CLAIMS:

3. The method of claim 1 wherein finely divided soy beans or soy flour comprises the crude vegetable protein.

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L6: Entry 23 of 26 File: USPT Feb 14, 1984

DOCUMENT-IDENTIFIER: US 4431629 A
TITLE: Method of producing an egg white substitute material

Abstract Paragraph Left (1):
Method of producing an egg white substitute material from soy protein. The method comprises extraction of a defatted soy bean material at a pH between about 6.0 and 10.5, separation, subjection of the supernatant to one or more ultrafiltrations and proteolytic hydrolysis of the supernatant or some fraction thereof to a DH between 1 and 8. The hydrolyzed soy material exhibits both a superior whipping or emulsifying ability and a good nutritional value, and it has no bitter taste.

Brief Summary Paragraph Right (6):
The first aspect of the present invention provides a method for producing an egg white substitute material based on soy protein, which method comprises extracting a defatted soy bean material with an aqueous medium at a pH in the range of from about 6.0 to about 10.5, separating the solid material from the supernatant and thereafter subjecting the supernatant to one or more ultrafiltration steps. At a convenient point during the procedure proteolytic hydrolysis is carried out on the supernatant or a fraction thereof, whereby the protein is hydrolyzed proteolytically to a DH in the range of from 1 to 8, the proteolytic activity being inactivated after the proteolytic hydrolysis.

Detailed Description Paragraph Right (1):
In all aspects of this invention the starting substance is a form of defatted soy material such as soy bean meal, flakes, flour, etc. the supernatant after the extraction normally contains the majority of the protein in the, defatted soybean material serving as the raw material. However, the defatted soy bean material may also serve as raw material if pretreated in such a manner that 50% of the protein present in the defatted soy bean material is extracted.

Detailed Description Paragraph Right (13):
In a preferred embodiment of the method according to the invention, defatted soy bean material is extracted with neutral tap water, to which has been added a base to provide a pH of around 8.0.

Detailed Description Paragraph Right (14):
In a preferred embodiment of the method according to the invention, the ratio between the weight of the aqueous medium used for the extraction and the weight of the defatted soy bean material, that is the extraction ratio, is selected in such a manner that ##EQU1##

Detailed Description Paragraph Right (28):
As has already been pointed out the term "defatted soy material" is intended to include defatted soy bean flour, defatted soy bean flakes, white flakes, meal or similar soy bean based materials.

Detailed Description Paragraph Right (40):
Soy protein isolate was produced from defatted white soy flour (Aarhus Oliefabrik A/S) according to the ultrafiltration process described by Hans Sejr Olsen in Lebensm.-Wiss. U. Technol. 11, 57-64 (1978). A series of enzymatic hydrolysates were prepared exactly as described in the already cited article in the ACS Symposium Series 92 (page 127). The series consisted of hydrolysates having DH of 0% (not enzyme treated, outside invention), DH of 1%, DH of 2%, DH of 3%, DH of 4%, and DH of 6% (the

hydrolysates with 1%.ltoreq.DH.ltoreq.6% being enzyme treated and in accordance with the present invention). The whipping expansions are shown versus DH in FIG. 6 of the accompanying drawings for both acid precipitated (results transferred from the above cited ACS paper) and for ultrafiltrated protein, prepared as above.

Detailed Description Paragraph Right (46):
Raw material: Defatted white soy flour (Aarhus Oliefabrik A/S).

Detailed Description Paragraph Right (48):
In Examples 2 to 4 all hydrolyses were performed with a proteolytic activity of 12 Anson units/kg of protein. In Examples 2 and 4, 11.5 kg of defatted white soy flour was extracted at pH of 8 using 108.5 l of water.

Detailed Description Paragraph Right (49):
Also, in all of the following Examples only 150 ml of protein solution was used for the determination of the whipping expansion due to the incredibly high whipping expansion of the protein hydrolysates.

Detailed Description Paragraph Right (50):
For the sake of comparison with the prior art methods, also results with proteins without hydrolysis and with acid precipitated proteins hydrolyzed to DH of 3% have been shown in the Tables in the following Examples, demonstrating the superior characteristics of the protein hydrolysate produced according to the invention.

Detailed Description Paragraph Right (58):
In Examples 5 to 8 all hydrolysis were performed with a proteolytic activity of 18 Anson units/kg of protein. In Examples 5 to 8, 50 kg of defatted soyflour was extracted at pH=8 using 500 l of water.

Detailed Description Paragraph Right (62):
In Table 8 the mass and volumes of phases obtained at the different operations are shown. 50 g of soy flour was extracted by use of 500 l H.sub.2 O at pH=8.0.

Detailed Description Paragraph Table (7):
TABLE 7 Defatted white soy flour Soy raw material (Aarhus Oliefabrik) Ultrafiltration module ROMICON-Hollow fibers (Alfa-Laval) Membrane area 4.8 m.sup.2 Membrane Type PM30 Cut-off-value: 30.000 Enzyme Alcalase .RTM. 0.6 L

CLAIMS:

1. In a method for producing an egg white substitute by water extracting defatted soybean material at a pH in the range of about 6-10.5, then subjecting the resulting extract to a hydrolyzing agent to effect proteolytic hydrolysis, with or without removal of the solid materials therefrom, and inactivating the hydrolyzing agent, the improvements which comprise:

hydrolyzing said extract to a degree of hydrolysis in the range of from 1 to 8 thereafter inactivating the hydrolyzing agent; and

removing solid materials from the extract, if not previously done, to produce therefrom a supernatant; then

subjecting the hydrolyzed supernatant to ultrafiltration, and thereafter recovering the egg white substitute from the retentate.

4. A method according to claim 1, wherein the defatted soy bean material is extracted at a pH of around 8.0.

5. A method according to claim 1, wherein the ratio between the extraction ratio weight of the aqueous medium used for the extraction to the weight of the defatted soy bean material is ##EQU4##

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L6: Entry 24 of 26 File: USPT Apr 13, 1982

DOCUMENT-IDENTIFIER: US 4324805 A
TITLE: Method of producing soy protein hydrolysate from fat-containing soy material, and soy protein hydrolysate

Abstract Paragraph Left (1):
A method for producing soy protein hydrolysate involving water washing fat-containing soy material at a pH of 3.5-5.5 thereby partially defatting the soy material; then hydrolyzing the partially defatted soy material with a proteolytic enzyme in the presence of water and a base to a DH in the range of 1-20; and recovering the aqueous soy protein hydrolysate from soy derived oil and solids in the hydrolysis mixture. The oil is recovered from the wash water and from the hydrolysate mixture.

Brief Summary Paragraph Right (1):
The Invention relates to a method for producing soy protein hydrolysate from fat-containing soy material, and to the soy protein hydrolysate.

Brief Summary Paragraph Right (2):
Soy protein hydrolysate is a material of growing importance, for example, in the food industry. Thus, it can be used as one of the main constituents in brines for meat pumping in order to enrich the protein content thereof, as a constituent in soy milk in order to enrich the soy milk with protein, and as a protein enriching agent used as an additive for both acid and neutral soft drinks.

Brief Summary Paragraph Right (3):
A method for production of soy protein hydrolysate from soy beans that had been defatted by extraction with organic solvents is known, being described, for example, in Fifth International Congress of Food Science & Technology, Abstract of paper 3B-14, "Enzymatic hydrolysis of soy protein. Processing developments and applications in low pH foods." However, the above referenced process for production of soy protein hydrolysate from defatted soy beans is not believed to be suited to fat-containing soy materials.

Brief Summary Paragraph Right (4):
Herein and in the accompanying claims as well, the term "fat-containing soy material" is used generically to include full-fat and partially defatted soy flour, ground whole soy beans, crushed soy beans and similar soy materials.

Brief Summary Paragraph Right (5):
Fat-containing soy material, especially full-fat soy flour, is available in huge amounts in industrially unsophisticated areas of the world.

Brief Summary Paragraph Right (6):
For commercially feasible production of a refined soy protein hydrolysate product from fat-containing soy materials as a starting material, a concomitant recovery of the fat content is important. Usually the recovery of soy oil from soy beans is carried out by extraction with organic solvents, generally a hexane extraction. However, the solvent extraction process involves recovery of solvent by fractional distillation and distillation equipment requires a relatively high level of capital investment. Furthermore, the solvent process is not ideal from an environmental point of view, especially since solvents used for the extraction ordinarily are highly flammable. Moreover, the solvent extraction process is so elaborate as to be poorly suited to use at production sites in developing countries.

Brief Summary Paragraph Right (7):
Thus, a need exists for a method for treatment of a fat-containing soy material well suited for production sites of a primitive nature, a method which results in an organoleptically acceptable soy protein hydrolysate and considerable recovery of the soy oil and other valuable materials present in the full fat-soy flour.

Brief Summary Paragraph Right (8):
The method for production of soy protein hydrolysate from fat-containing soy material according to the invention comprises hydrolyzing a partially defatted solid soy material, obtained by washing fat-containing soy material such as soy flour with an aqueous medium at an acid pH, the partially defatted soy material being treated with a proteolytic enzyme in the presence of water and a base to hydrolyze same to a DH in the range of from 1 to 20 and thereafter deactivating the enzyme, whereafter the aqueous hydrolysate is separated from the oil and solids.

Brief Summary Paragraph Right (10):
Advantageously, the method of producing soy protein hydrolysate from fat-containing soy material according to the invention comprises washing the fat-containing soy material in an aqueous medium at a pH, preferably in the range of from 4.2 to 4.5 (Operation I). The wash water from Operation I is separated into an oil phase and a waste water phase (Operation II), the washed, partially defatted solid soy material from Operation I being then introduced into a hydrolysis reaction vessel, to which water, a proteolytic enzyme, and base are added. In the hydrolysis reactor, the partially defatted soy material from Operation I is hydrolyzed at a relatively constant pH to a degree of hydrolysis (DH) in the range of from 1 to 20 (Operation III), whereafter the proteolytic activity is inactivated by acid addition to a pH of 4.0 or less. The slurry from the hydrolysis of Operation III is separated into an oil phase, an aqueous hydrolysate phase and a sludge phase (Operation IV). The sludge phase from Operation IV is collected as Product A, the oil phases from Operations II and IV are combined as Product B and the aqueous soy protein hydrolysate phase from Operation IV is collected as Product C.

Brief Summary Paragraph Right (11):
The invention also relates to the soy protein hydrolysates produced by the method of the invention.

Brief Summary Paragraph Right (12):
The present invention provides a method well suited for production sites of a primitive nature capable of recovering a good yield of valuable soy protein hydrolysate without bitterness, without soy flavor and without any disadvantageous properties originating from the soy fat, the Product C, around 60% of the oil content in the initial fat-containing soy material as a separate oil phase, Product B, and a precipitate or sludge from the hydrolysis, which can be used either as a high grade fodder or for the starting material in a renewed hydrolysis step, Product A.

Brief Summary Paragraph Right (13):
Surprisingly, it has been found that the soy protein hydrolysate of the invention can be fully acceptable from an organoleptic point of view and also that the oil phase does not turn rancid during the recovery thereof.

Detailed Description Paragraph Right (1):
Referring now to FIG. 1, a fat-containing soy material 25, which should of course be obtained from the soy plant without formation of off-flavors e.g., soy flour by crushing soy beans, is washed (extracted) with water 35 in washer 30 (Operation I). Sufficient acid 45 is introduced initially into washer 30 until the pH in the wet soy material is in the range of from 4 to 4.5. By pH turns out to be essentially constant, even if large amounts of water are later added. The soy material is washed until it has a bland taste, and until all soluble materials (at pH 4 to 4.5) are removed. A stepwise washing operation in which each wash step includes separation of the liquid and the solid phases may be used. The washing operation may be countercurrent, or with fresh or recycled wash water in each wash step. At least four wash steps are preferred.

Detailed Description Paragraph Right (2):
If a liquid/solid ratio of 10:1 is used in each wash step, the separation from

Operation I can be carried out by means of decanter centrifuges or other types of separators. Suitable types of equipment that may be used are, for example, basket centrifuges, continuous or batchoperating countercurrent extractors or press-equipment. From the washing step of Operation I, a partially defatted and washed soy material 11 is removed. Furthermore, the total amount of wash liquid 12 is recovered and in separator 40 (Operation II), this liquid is separated into an oil phase 13 and an oil free wash water phase 14, which may be regarded as waste water.

Detailed Description Paragraph Right (3):
The partially defatted and washed soy material 11 is transferred to a hydrolysis reaction tank 50 equipped with a stirrer, thermometer and pH-electrodes connected to a titrator, wherein hydrolysis (Operation III) takes place. Water 55 is added to the soy material 11 until the protein concentration in the reaction mixture is in the range of from 6 to 10% (N.times.6.25). Base 75, e.g., NaOH is added. The temperature of the reaction mixture is adjusted to 50.degree.-55.degree. C. and the proteinase 65, preferably "ALCALASE" is added. If the hydrolysate is intended for nutritional purposes, a food grade preparation of the proteolytic enzyme is used in amounts sufficient to carry out hydrolysis in around two hours.

Detailed Description Paragraph Right (6):
The finished hydrolysate 15 is then passed to separator 60 wherein it is separated (Operation IV) into an oil phase 17, a soy protein hydrolysate 16 and a sludge phase 18, the last containing insoluble protein, polysaccharides and residual amounts of oil. Preferably, a three-phase-centrifuge is used for separator 60, but the combination of a solids ejecting centrifuge followed by a liquid separator is also usable.

Detailed Description Paragraph Right (10):
The oil-free aqueous phases 16 and 22 from separators 60, 80 of Operations IV and VI are combined to become the raw soy protein hydrolysate of product 300. Product 300 then may be carbon treated, concentrated and dried, as described in, for example, U.S. Pat. No. 4,100,024.

Detailed Description Paragraph Right (12):
600 g of full-fat soy flour 25 (Nutridan TF-100-L from Dansk Soyakagefabrik A/S) having the following composition:

Detailed Description Paragraph Right (13):
To 666.5 g of the partially defatted soy flour solids taken off the centrifuge the pH value of which was 4.35, was added 39.6 ml of 4 N NaOH to reach pH 8.0, and 1282 g of water 55 to dilute the suspension to approximately 8% protein (N.times.6.25). The mixture was heated to 50.degree. C. in a water bath. Then 3.20 g of "ALCALASE" 0.6 (0.65 Anson units per gram) was diluted to 50 ml with water and added to the suspension containing the partially defatted soy flour. Thereby an enzyme activity of 13.1 Anson units per kg protein was obtained.

Detailed Description Paragraph Right (15):
The hydrolysis mixture was then centrifuged (Operation IV) in a laboratory centrifuge (Beckmann model J-6B) at 3000 x g for 15 minutes, and 1500 g of a centrifugate containing both oil and protein hydrolysate, and 554 g of sludge was collected. The sludge phase 18 was washed with 1500 g of water and centrifuged as above to yield 1500 g of centrifugates and 500 g of sludge being Product A. The results obtained after performance of the above described Operations III, IV and V are shown in Table 3.

Detailed Description Paragraph Right (16):
After skimming the oil phases therefrom, the two centrifugates from Operations IV and VI were combined and adjusted to pH=5 by 4 N NaOH (amount not determined), then activated carbon (BGN from Lurgi Apparate-Technik) was added in an amount of 0.2% of the total volume of hydrolysate. After stirring for 30 minutes at 50.degree. C. the activated carbon was removed by filtration through a glass fibre filter (Watman glass fibre GF/F) which had previously been washed with 5 liters of deionized water, in order to remove off-flavors from the filter. The filtrate was adjusted to pH=6.5 and diluted to 4% protein (N.times.6.25) before evaluation by means of a trained taste panel consisting of 14 persons. This product hydrolysate was compared with a sample produced from defatted flakes, as described in e.g., Fifth International Congress of

Food Science & Technology, Abstracts of paper, 3B-14, "Enzymatic hydrolysis of soy protein. Processing development and applications low pH foods". A triangle-taste-evaluation resulted in seven right answers and seven wrong answers, indicating that a taste difference could not be demonstrated. (The word "Watman" is a Trade Mark.)

Detailed Description Paragraph Right (17):
20 kg of full-fat soy flour (Nutridan TF-100 L from Dansk Soyakagefabrik A/S) having the composition indicated in Example 1 was stepwise washed at pH=4.2 using 4.times.180 liters of water of 15-20.degree. C. (Operation I), acid being introduced into the first wash step only. Each wash step includes stirring of the solid phase and water followed by centrifugation in a decanter centrifuge (Alfa-Laval NX 310-B). The sludge content in the centrifugate (determined after centrifugation of 10 ml in a graduated tube) was 2-4%. Therefore, the centrifugate was re-centrifuged in a solids ejecting centrifuge Westfalia (SB 7-35-076). The results are shown in Table 4. The centrifuge indicated in Table 4 was the centrifugate from the Westfalia centrifuge and the sludge was the combined (total) sludge from the decanter and the solids ejecting centrifuge (Operation I). The total combined 630 liters of centrifugate were separated into 2.8 kg of oil phase and 627 kg of an oil-free aqueous phase using a Westfalia centrifuge of type LG 205-2. The results obtained are shown in Table 5. (The word "Westfalia" is a Trade Mark.)

Detailed Description Paragraph Right (19):
To 41 kg of the partially defatted soy flour was added 46 kg of water to dilute the sludge to about 6.75% protein. 685 ml of 4.8 N NaOH was added to adjust the pH to 8.0. The mixture was stirred and heated to 55.degree. C. in a tank with a heating mantle. 118 g of "ALCALASE 0.6 L (0.65 Anson units/g) was diluted to 5 liters with cold water and added to the suspension. During the hydrolysis, pH was kept constant at 8.0 by addition of 4.8 N NaOH using the pH-STAT-technique. A DH of 10% was reached after 133 minutes when 843 ml of 4.8 N NaOH had been consumed. Immediately thereafter, 1887 g DL-malic acid was added to give a pH of 4.0. The suspension was stirred for 30 minutes in the heated tank to inactivate the enzyme (Operation III).

Detailed Description Paragraph Right (21):
The sludge was washed with 70 liters of water (Operation V) and separated into 73 liters of sludge Product A and 45 liters of wash liquid which in turn was separated into 43 liters of oil-free aqueous phase and 66 g of oil (Operation VI). Results obtained during the recovery of soy protein hydrolysate are shown in Table 7.

Detailed Description Paragraph Left (1):
was stepwise washed at pH 4.2. Each step includes a stirring of the solid phase and water for 30 minutes followed by a centrifugation at 3000.times.g for 20 minutes in a laboratory centrifuge (Beckmann model J-6B). Results obtained from this multi-step washing procedure (Operation I) are shown in Table 1, together with the composition of protein (N.times.6.25), fat and total dry matter of the partially defatted soy flour and the combined centrifugates from the four steps. Based on these results the mass balance and yields are shown in Table 2. (The word "Nutridan" is a Trade Mark, as is the word "Beckmann").

Detailed Description Paragraph Table (1):
TABLE 1

CENTRIFUGATE AND SOLID PHASE RELATED TO OPERATION I Combined or 1. step 2. step 3. step 4. step final

Full-fat soy flour (g) 600 -- -- -- -- 6 N HCl (ml) 34.5 0 0 0 -- Water (g) 6000 6000 5000 5000 -- Centrifugate: Mass (g) 5160 5300 5000 5000 20460 Protein conc., N .times. 6.25 (%) 0.25 0.07 0.07 0.04 0.10 Dry matter (%) 2.22 0.49 0.23 0.15 0.78 Fat (%) not determ. not determ. not determ. not determ. 0.20 Solid phase: Mass (g) 1050.6 991.4 1032.0 1009.7 1009.7 Protein conc. N .times. 6.25 (%) not determ. not determ. not determ. 23.9 23.9 Dry matter (%) " " " 40.7 40.7 Fat (%) " " " 8.2 8.2

Detailed Description Paragraph Table (2):
TABLE 2

MASS BALANCE AND YIELDS RELATED TO OPERATION I Partially Full-fat soy Combined centri- defatted soy flour fugate flour

Total mass (g) 600 20460 1009.7 Mass of dry matter (g) 570.1 159.6 410.9 Yield (%) 100 28.0 72.1 Mass of pro- tein (g) 259.1 20.5 241.3 Yield (%) 100 7.9 93.1 Mass of fat (g) 123 40.9 82.8 Yield (%) 100 33.3 67.3

Detailed Description Paragraph Table (3):

TABLE 3 RESULTS OBTAINED AFTER PERFORMANCE OF OPERATIONS III AND IV Yield of fat % Yield of Based protein on par- % tially Based on defat- partially ted defatted flour/ flour/ based Mass of based on on full- Process step fraction, % Pro- full-fat fat and fraction g tein flour % Fat flour Operation III. Partially defatted soy 666.5 23.9 100/93.1 8.2 100/67.3 flour After hydrolysis 2117.8 7.5 100/93.1 2.6 100/67.3 Operation IV. Centrifu- gate 17 + 1500 4.3 40.6/37.8 1.2 32.7/ 16 22.2 Sludge 18 554 not analysed not analysed Operation V Centrifugate 1500 not Analyzed Not Analyzed 21 + 22 Product A 500 14.3 44.9/41.8 7.3 66.8/45.0

Detailed Description Paragraph Table (4):

TABLE 4 CENTRIFUGATE AND SOLID PHASE RELATED TO OPERATION I Combined or 1. step 2. step 3. step 4. step final Full-fat soy flour (kg) 20.0 -- -- -- -- 6N HCl (kg) 1.3 -- -- -- -- Water (kg) 180.0 180 180 180 720 Centrifugate: Mass (kg) 155 160 160 155 630 Protein (% N .times. 6.25) 0.38 0.13 0.13 0.13 0.25 Dry matter (%) 3.30 0.43 0.50 0.21 0.96 Fat (%) 1.23 0.16 not determ. 0.20 0.50 Solid phase: Mass (kg) 59.3 57.4 51.8 51.5 51.5 Protein (% N .times. 6.25) 16.44 16.0 15.06 14.19 14.19 Dry matter (%) 27.51 27.7 22.95 21.77 21.77 Fat (%) 4.12 not determ. not determ. 1.80 1.80

Detailed Description Paragraph Table (7):

TABLE 7 RESULTS OBTAINED AFTER PERFORMANCE OF OPERATIONS III, IV, V, AND VI Yield of protein % Yield of fat based on Based on partially based on partially Based on Operation and Mass of frac- defatted full-fat defatted full-fat fraction tion kg % protein flour flour % fat flour flour Operation III 11 41 14.19 100 84.6 1.80 100 22.4 15 95.5 6.56 -- -- (1.67) -- -- Operation IV 17 0.084 2.63 0.04 0.03 60.4 6.9 1.5 16 34 4.38 25.6 21.7 -- -- -- 18 50 7.63 65.6 55.5 not ana- lyzed -- -- Operation V 10 A 73 2.75 34.5 29.2 not ana- -- -- lyzed 11 45 1.88 14.5 12.3 not ana- -- -- lyzed Operation VI 21 0.066 2.53 0.03 0.02 61.5 5.5 1.2 22 43 1.81 13.4 11.3 -- -- --

() means that the figure is unrealistic.

Detailed Description Paragraph Table (8):

TABLE 8 COMPOSITION AND YIELDS OF PRODUCT A, B AND C BASED ON FULL-FAT SOY FLOUR Component A B C Protein % 2.75 1.5 2.99 Yield % 29.2 0.5 33.0 Dry matter 10.3 65 4.5 Yield % (84.2) 10 22.9 Oil % not determ. 60 -- Yield % not determ. 43.4 -- () means that the figure is unrealistic.

CLAIMS:

1. An organic solvent free method for producing soy protein hydrolysate from soy material containing recoverable amounts of oil which comprises:

water washing such a soy material at a pH of 3.5-5.5 thereby partially defatting the soy material and extracting water soluble substances therefrom; then

hydrolyzing the partially defatted soy material at pH 6-12 with effective amounts of a proteolytic enzyme in water to a DH in the range of 1-20; and, thereafter,

separating the aqueous soy protein hydrolysate from soy derived oil and solids in the hydrolysis mixture.

4. The method of claim 1 wherein oil released from the soy material into the wash water is separated out therefrom, and oil released from the partially defatted soy material into the hydrolysate mixture is separated out therefrom, the wash water oil and the hydrolysate oil both being recovered.

9. A method for production of soy protein hydrolysate and oil from soya material containing recoverable amounts of oil which comprises:

water washing such a soy material at pH 3.5-5.5, thereby partially defatting the soy material; and extracting water soluble substances therefrom; then

separating the wash water into an oil phase and an aqueous phase;

hydrolyzing the partially defatted soy material at pH 6-12 with effective amounts of a proteolytic enzyme in water to a DH of 1-20; thereafter deactivating the proteolytic enzyme; and then

separating the hydrolysis mixture into a sludge phase, an aqueous phase and an oil phase,

the oil phases from the hydrolysis mixture and from the wash water being the oil product, and the aqueous phase from the hydrolysis mixture being the soy protein hydrolysate product.

10. The method of claim 9 wherein the sludge phase is water washed and wherein the washings therefrom are separated into oil phase and aqueous phase for recovery of additional oil and protein hydrolysate product therefrom.